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Source, impact and removal of malodour from soiled clothing

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ABSTRACT

Static headspace – multi-capillary column – gas chromatography – ion mobility spectrometry (SHS-MCC-GC-IMS) has been applied to the analysis of malodour compounds from soiled clothing (socks and T-shirts), pre- and post washing, at low temperature (20 °C). Six volatile compounds (VCs) (i.e. butyric acid, dimethyl disulfide, dimethyl trisulfide, 2-heptanone, 2-nonanone and 2-octanone) were identified. After sensory evaluation of soiled garments they were subjected to laundering with non-perfumed washing powder. The efficiency of the laundering process was evaluated by determining the reduction of each detected volatile compound (VC) post-wash (damp) for socks and T-shirts; VC concentration reductions of between 16 and 100% were noted, irrespective of sample type. Additionally the T-shirt study considered the change in VC concentration post-wash (dry) i.e. after the drying process at ambient temperature. Overall VC concentration reductions of between 25 and 98% were noted for T-shirt samples pre-wash to post-wash (dry). Finally, a potential biochemical metabolic pathway for the formation of malodour compounds associated with bacteria in axillary sweat is proposed.

1. Introduction

Laundry has been an important domestic activity in the whole of human life. It is not known when and where human beings first started to wash their clothes. In the past laundry was commonly carried out in rivers and streams by hand and scrubbing with soap; this process is still used in developing countries today. Laundry washing is a means of removing dust and dirt from clothing and also of getting rid of malodour i.e. the presence of unpleasant smell on clothes. Nowadays advanced washing machines with several washing programmes (wash cycles) and effective detergents make washing easier and more efficient.

Personal malodour arises from the production of VCs generated by the action of microorganisms in breaking down the components of sweat, human skin cells and secretion substances from the glands. Characteristic malodour VCs that can arise from these processes include ammonia and hydrogen sulfide as well as short chain fatty acids; its occurrence can be a cause of personal embarrassment. As well as VCs generated from the human body, they can also arise from external sources, such as, the washing machine, as well as the drying environment. However, the characteristic malodour generated from laundries, which can be detected just after washing, can be due to poor hygiene in laundering resulting from microbial fabric damage and biofilm build up inside the washing machine [1]. Traditionally in Europe, laundry has been washed at a high temperature (>60 °C). However, for environmental reasons, lower temperatures are now encouraged. At low temperature the generation of malodour is more common [1,2]. Detergent manufacturers have been designing detergents for low temperature washing (i.e. between 15–20 °C) and these are now available on the European market. The addition of perfumes in laundry detergents and fabric conditioners are used to neutralize unpleasant odours on clothing, which are not reactively removed in the washing process. It is apparent, by the range of products on supermarket shelves and their associated marketing that household consumers would be less satisfied with their garments if they were laundered with non-perfumed detergents. Modern detergents have been formulated as complex mixtures with various types of surfactants (ionic and non-ionic), bleach, enzymes as well as essential oils. Generally, surfactants in detergents are designed to remove dirt, in to the aqueous phase, while perfumes tend to remain on the garment.

Numerous factors have been identified for the formation of malodour in laundries, such as, the drying environment [3,4], bacterial colonisation (biofilms) [4,5] and metabolism of micro-organisms [6] as well as human odour [7]. However, limited studies have been carried out to investigate the bacterial colonisation (biofilms) in washing machines [4,5]. In household washing machines, microbial survival followed by biofilm formation occurs due to soiled garments and poor water treatments [5]. A number of microbe species have been isolated and identified from the biofilm within

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washing machines [1,4,5]. Among the garment and water carrying parts of washing machines "hot spot" biofilm formation have been discovered (i.e. product drawer, sump and rubber seal) [4]. Moreover a recent study [4] showed a possible link that contamination of fabrics occurs from the washing machines itself, based on the identification of the same bacteria in the washing machine rubber seal and on fabrics. According to this study potential VC markers for both high levels of bacteria and malodour in washing machines are dimethyl disulfide and dimethyl trisulfide.

In this paper, characteristic malodour marker VCs were determined using static headspace – multi-capillary column – gas chromatography – ion mobility spectrometry (SHS-MCC-GC-IMS). This analytical technique was selected over gas chromatography mass spectrometry due to its enhanced detection of sulfur-based compounds, speed of analysis and multi-dimensional detection capabilities [8]. The influence of the washing process on the reduction of VCs has been evaluated from pre-wash to post-wash on pre-selected soiled clothing items at low temperature. The washing process was evaluated using a developed scaled-down laundry approach using non-perfumed washing powder. The clothing items, namely socks and T-shirts, were worn by volunteers during either normal or extended physical activity, respectively. The study was extended to take in to account the drying process in the extended physical activity study.

2. Experimental

2.1. Chemicals/reagents

Acetone (CAS 67-64-1,≥99.9%), ammonia (CAS 1336-21-6, 28% NH3 in H2O,≥99.99%), butyric acid (CAS 107-92-6,≥99%), dimethyl disulfide (CAS 624-92-0,≥98%), dimethyl trisulfide (CAS 3658-80-8,≥98%), 2-heptanone (CAS 110-43-0, ≥98%), 2-nonanone (CAS 821-55-6,≥98%), and 2-octanone (CAS 111-13-7, ≥98%) were all purchased from Sigma–Aldrich (Dorset, UK). Stock solutions (10,000 ppm) were prepared using acetone. Milli-Q water of conductivity 18.2 MΩ-cm was produced by a direct QTM Millipore system 165 (Molsheim, France) and was used in all dilution steps.

Sock samples (74% cotton, 19% polyester, 5% nylon and 2% lycra) and 100% polyester white sport T-shirts (Nike) were obtained from a local retail outlet (Newcastle, UK). Headspace (20 mL) crimp-cap vials and magnetic caps were purchased from Sage Analytical Ltd. (Lancashire, UK). Nylon Fire Bags (250 mm × 375 mm) were obtained from Crime Scene Investigation (Woburn Sands, UK) and were used for collection and storage of the soiled fabric samples. A leading granular laundry detergent (non-perfumed and perfumed) were obtained from the Procter & Gamble Technical Centre, Newcastle upon Tyne.

2.2. Instrumentation

A static headspace – multi-capillary column – gas chromatography – ion mobility spectrometer (SHS-MCC-GC-IMS) manufactured by G.A.S.-Gesellschaft für Analytische Sensorsysteme mbH (Dortmund, Germany), was used [8]. The SHS-MCC-GC-IMS was fitted with an automatic sampler unit (CTC-PAL; CTC Analytics AG, Zwingen, Switzerland) and a heated gas-tight syringe. A multi-capillary column (MCC) (Multichrom, Novosibirsk, Russia) was used for the chromatographic separation. The MCC comprised a stainless steel tube, 20 cm × 3 mm ID, containing approximately 1000 parallel capillary tubes, 40 μm ID, coated with 0.2 μm film thickness of stationary phase i.e. OV-5. Atmospheric pressure ionisation is generated by a Tritium (^{3}H) solid state bonded source (β-radiation, 100–300 MBq with a half-life of 12.5 years). The IMS has a drift tube length of 50 mm. Separation in the IMS drift tube is achieved by applying an electric field of 2 kV to the ionized volatiles in a pulsed mode using an electronic shutter opening time of 100 μs. The drift gas was N_2 (99.998%) with a drift pressure of 101 kPa (ambient pressure). Samples were run under the following operating conditions: incubation conditions (time, 5 min; and, temperature, 95 °C); MCC-IMS conditions (syringe temperature, 85 °C; injection temperature, 80 °C; injection volume, 1.5 mL; column temperature, 35 °C; and, carrier gas flow rate, 10 mL/min); and, IMS conditions (temperature, 60 °C; and, drift gas flow rate, 500 mL/min) for butyric acid, dimethyl disulfide and dimethyl trisulfide whereas for 2-heptanone, 2-nonanone and 2-octanone the incubation conditions were as follows: (time, 5 min; and, temperature, 95 °C); MCC-IMS conditions (syringe temperature, 85 °C; injection temperature, 80 °C; injection volume, 1.5 mL; column temperature, 35 °C; and, carrier gas flow rate, 150 mL/min); and, IMS conditions (i.e. temperature, 45 °C; and, drift gas flow rate, 500 mL/min). All data was acquired in the positive ion mode and each spectrum is formed with the average of 42 scans. All data are processed using the LAV software (version 2.0.0, G.A.S). The software package enables both two- and three- dimensional data visualisation plots.

A tergotometer (Copley Scientific, Nottingham, UK) was used to simulate the washing machine. The tergotometer contained eight stainless steel vessels, each with a capacity of 1000 mL. The temperature within the stainless steel vessels of the tergotometer was controlled by a water circulatory heating system. The temperature was adjustable in the range ambient to 70 °C. Each stainless steel vessel was capable of being stirred within the range 50–200 rpm. Sub-samples of socks and T-shirt were added to the tergotometer in the approximate ratio of 2.5 g: 300 mL (fabric: water).

2.3. Procedure for sock analysis

Eight healthy volunteers (6 males and 2 females) were selected to participate in this study. Each participant received a pair of new socks and a wear protocol; each sock was enclosed in a uniquely coded sample bag i.e. right sock and left sock. Participants were not allowed to apply odorous products during the study i.e. deodorant or moisturiser. The wear protocol was as follows: each foot was rinsed thoroughly with tap water and then dried. Participants then wore the socks over a minimum period of 10 h during one day in a specified type of footwear i.e. shoes. After use the participants transferred each sock into the uniquely coded bag, which was sealed and stored overnight in a dark place. The sample containing bags were then returned to the investigator the following day. Each sample was olfactory graded according to a numerical scale ranging from 0 (no malodour) to 10 (malodorous).

The socks were then sub-sampled in three distinct areas i.e. toe, ball and heel by taking approximately 2.5 g of fabric. The sub-samples were then placed into 20 mL headspace vials and closed with a magnetic cap prior to SHS-MCC-GC-IMS analysis. Unworn socks were prepared as blank samples and analysed using the same methods; this allowed extraneous VCs to be identified and eliminated from future data treatment. Samples were washed according to the developed Tergotometer washing protocol using non-perfumed washing powder. After washing, the sock subsamples were manually wrung out using fingers and each placed in a 20 mL vial for analysis as post-wash (damp) samples using SHS-MCC-GC-IMS.

2.4. Procedure for T-shirt analysis

Prior to wearing the T-shirts they were washed in a domestic washing machine (Servis, 1200 rpm) that itself was pre-cleaned. Pre-cleaning of the washing machine was done using Dr Beckmann Service-it Deep Clean washing machine cleaner by following the supplier instructions and washing at 60 °C for approximately 1 h.

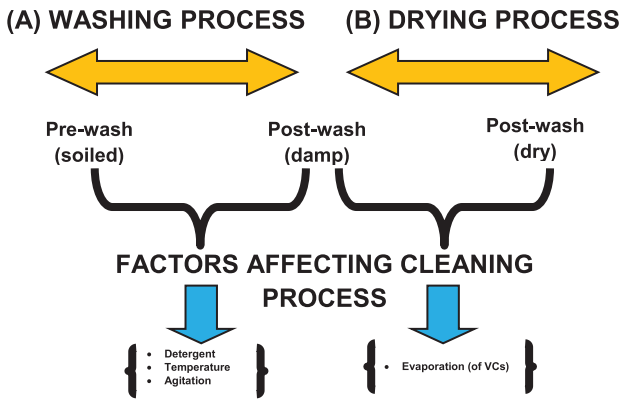

Fig. 1. Factors influencing the cleaning process in laundering.

This was done to prevent cross contamination of the T-shirts from the washing machine [4]. Then, all T-shirts were laundered at 40 °C, 1200 rpm spin for one hour with non-perfumed washing powder. After the washing process, each T-shirt was air-dried indoors and overnight at 22 °C and then stored in an individual nylon bags prior to use.

Nine healthy adult volunteer males (aged from 20–31 years) were selected for this investigation. They were all taking part in an indoor 5-a-side football tournament (held in Newcastle upon Tyne, 26th March 2014). Participants wore the T-shirts for approximately 2–3 h (in between each match participants had a 10 min break). After the end of the tournament, the participants transferred their T-shirts in to a uniquely coded bag; in addition, an unworn T-shirt was retained as a blank sample. All samples were kept in a refrigerator (4 °C) until the analyses were done using SHS-MCC-GC-IMS. In addition, a physical description of the T-shirts as well as their olfactory malodour grading was recorded post-use (Table 1).

A sub-sample from the under arm area of each T-shirt was removed; each sub-sample weighed between 2.4 g–3.1 g. The sub-samples were placed into 20 mL headspace vials and closed with a magnetic cap prior to SHS-MCC-GC-IMS analysis. Samples were washed according to the developed Tergotometer washing protocol. After washing, the T-shirt samples were placed in 20 mL vials for analysis. Subsequently, all samples were air-dried overnight at ambient temperature (20 °C) and collected for re-analysis.

3. Results and discussion

Laundering of soiled garments can be considered to be composed of two distinct but different processes: (A) the washing process and (B) the drying process (Fig. 1). In the washing process three distinct, but specific activities, can be identified: the pre-wash (involving the selection and addition of soiled garments to the washing machine); the washing process itself i.e. addition of detergent (powder or liquid), water and mechanical agitation; and, the post-wash (damp) state in which the garments are left inside the washing machine until removed. The washing process can therefore be considered to consist of a physical process, involving temperature and agitation, and a chemical process, based on the composition of the detergent (powder or liquid) in the presence of water and the soiled garments. In the second phase (B) it is the processes that take place between the post-wash (damp) and post-wash (dry) that are important; specifically, within the drying process, physical evaporation of VCs and water takes place as a direct result of an increased ambient temperature. Of significant importance in this study are the physical and chemical processes that occur in both the washing (A) and drying (B) processes and their influence on malodour, as evidenced by the determination of VCs.

3.1. Quantitative analysis using SHS-MCC-GC-IMS

Previous research from this group [7] investigated the use of SHS-MCC-GC-IMS for the separation and analysis of 32 VCs of known importance in malodour studies. In this previous study [7] 6 sets of experimental operating variables were investigated based mainly on MCC-GC conditions (i.e. column, column temperature and carrier gas flow rate) and the IMS condition (i.e. IMS temperature). As a result 2-dimensional maps, of retention time versus drift time, of the separated VCs were generated that illustrated the clarity of separation, in terms of resolution, achievable using SHS-MCC-GC-IMS. The 2-dimensional maps are also an important diagnostic tool in terms of their demonstration of the specific molecule characteristics of each VC i.e. whether a monomer, dimer or trimer was formed, as well as generating a database for future compound identification.

In this work it was possible to identify 6 VCs using SHS-MCC-GC-IMS as being significant, specifically, butyric acid, dimethyl disulfide, dimethyl trisulfide, 2-heptanone, 2-nonanone and 2-octanone. Two-dimensional maps of the 6 VCs are shown in Fig. 2(A + B). Table 2 summarises the key parameters investigated for each VC and includes details of their specific compound cluster (i.e. monomer, dimer or trimer) for quantitative analysis, retention time (s) and drift time (ms), the calculated normalised reduced ion mobility, the effective linear range (required for spiking experiments) and its equation and correlation coefficient, as well as details of the limits of detection and quantitation for each VC; the limits of detection varied between 0.3 ng for 2-nonanone to 74 ng for dimethyl disulfide with corresponding limits of quantitation of 1.0 ng for 2-nonanone to 248 ng for dimethyl disulfide. It is noticed how the effective linear range per VC is variable ranging from a narrow range (0–10 ng for 2-heptanone and 2-octanone) to a much wider range (0–6000 ng for dimethyl trisulfide); this had previously been reported by this group [8].

3.2. Influence of sample condition on SHS-MCC-GC-IMS recoveries

Several investigations have indicated that the ion mobility spectrometry signal can be affected by the moisture content of the sample [9–11]. As this will be an important aspect of this work a preliminary investigation was undertaken using unworn T-shirt sub-samples. This was investigated by the spiking of identified VCs on both pre-wash (dry) and post-wash (damp) unworn sub-samples. Four VCs were selected, as being representative of identified malodour compounds; specifically dimethyl trisulfide, 2-heptanone, 2-octanone and 2-nonanone. The unworn T-shirt sub-samples were placed in the 20 mL vial. Then, a 100 μL of a solution containing each VC was spiked directly onto the sub-samples. After spiking, the vial was immediately closed with a magnetic cap and analysed by SHS-MCC-GC-IMS.

The sub-samples ($n = 3$) were spiked within the linear range concentration of each VC i.e. (dimethyl trisulfide, 100 ng; 2-heptanone, 10 ng; 2-octanone, 10 ng; and, 2-nonanone, 50 ng). The following average percentage recoveries were obtained: pre-wash (dry): post-wash (damp); dimethyl trisulfide 39%: 32%; 2-heptanone 93%: 98%; 2-octanone 58%: 61%; and, 2-nonanone 44%: 35%, respectively. Statistical analysis, using a two-tailed paired *t*-test, was done to investigate whether there was any statistical difference between the VC signals on pre-wash (dry) and post-wash (damp) samples. The results indicate that there was no statistical significant difference (p-values < 0.05) in the recoveries between pre-wash (dry) and post-wash (damp), except for dimethyltrisulfide. It was

Table 1
Physical and sensory perception of whole T-shirts and their associated olfactory malodour grading.

Participant code	Physical and sensory perception of whole T-shirt	Odour description of whole T-shirt	Malodour grading*
M1	Sweaty, soiled	Mild malodour	5
M2	Sweaty, soiled	Mild malodour	5
M3	Dry, slightly soiled	Mild malodour	4
M4	Dry, slightly soiled	Mild malodour	4
M5	Sweaty, soiled	Mild malodour	4
M6	Sweaty, soiled	Malodour with strong acidic odour	8
M7	Sweaty, soiled	Mild malodour	5
M8	Sweaty, soiled	Mild malodour	6
M9	Sweaty, soiled	Mild malodour	5

* Malodour grading score: no malodour – odour grade 0; believe there is malodour – odour grade 2; there is a malodour – odour grade 4; malodour is strong – odour grade 6; malodour is very strong – odour grade 8; malodour is extreme – odour grade 10.

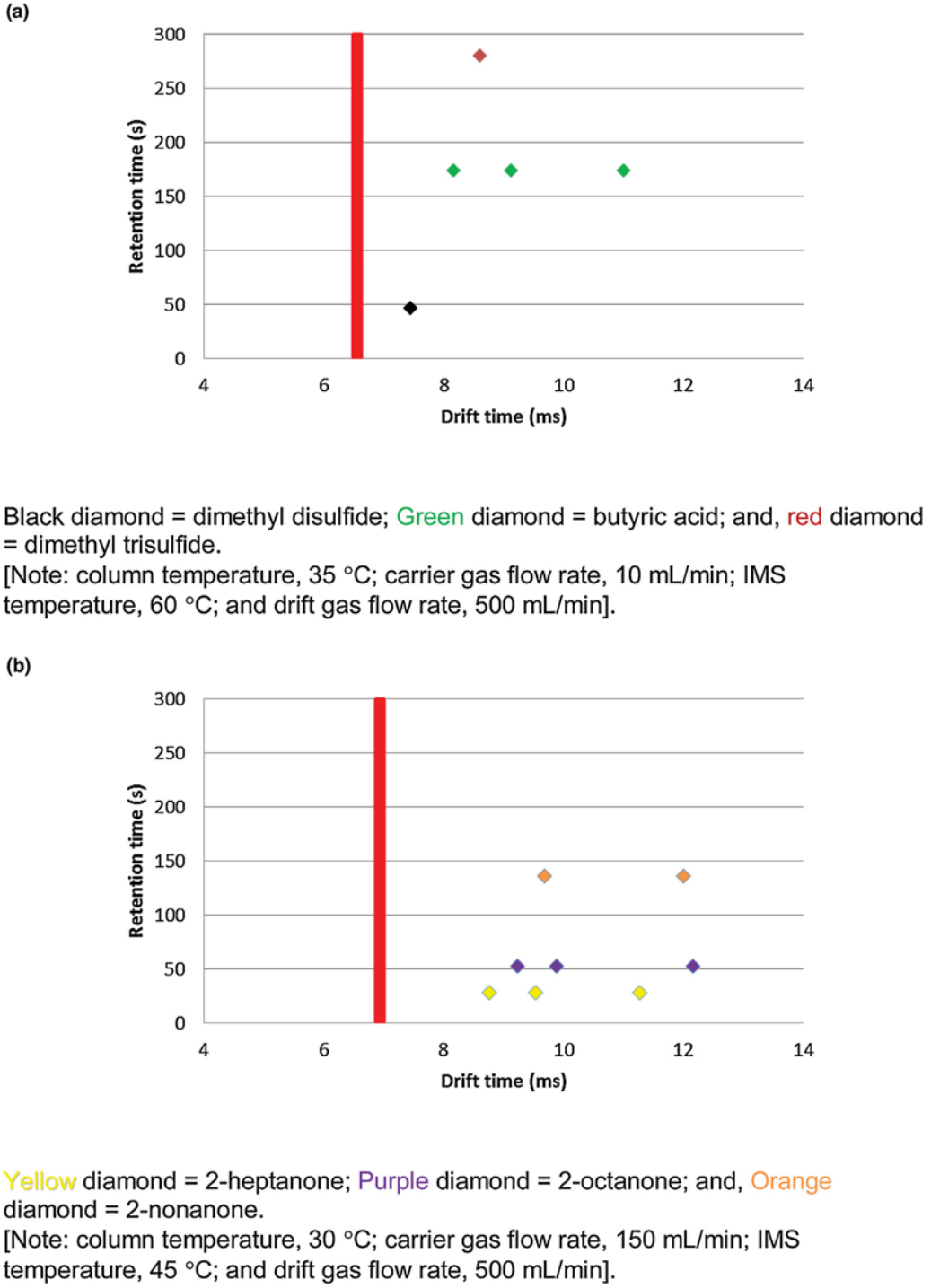

Fig. 2. Two-dimensional maps of the 6 VCs separated on an OV-5 MCC-GC column.

Table 2
Quantitative data for volatile compounds by SHS–MCC–GC–IMS.

Compound	Compound cluster[a]	Retention time (s) Mean ± SD (n = 3)	Drift time, ms (K_0 normalised reduced ion mobility, cm2 V^{-1} s^{-1}) Mean ± SD (n = 3)			Effective linear range (ng)	Equation for the effective linear range	Correlation coefficient, R^2	LOD (ng)	LOQ (ng)
			monomer	dimer	trimer					
Butyric acid	M + D + T	174 ± 2	8.2 ± 0.01 (1.87 ± 0.01)	9.1 ± 0.04 (1.67 ± 0.01)	11.0 ± 0.01 (1.39 ± 0.01)	30–2000	$Y = 0.0003x + 0.0098$	0.9947	9.0	30
Dimethyl disulfide	M	46.4 ± 0.6	7.5 ± 0.01 (2.05 ± 0.01)	ND	ND	250–6000	$Y = 0.0006x - 0.1293$	0.9900	74	248
Dimethyl trisulfide	M	280 ± 6	8.6 ± 0.04 (1.77 ± 0.01)	ND	ND	9–100	$Y = 0.0065x - 0.0283$	0.9812	3.0	9.0
2-Heptanone	M + D + T	28.2 ± 1.6	8.8 ± 0.03 (1.22 ± 0.01)	9.5 ± 0.01 (1.12 ± 0.01)	11.3 ± 0.01 (0.95 ± 0.01)	2–10	$Y = 0.1886x + 0.0101$	0.9847	0.7	2.3
2-Nonanone	D[b]	136 ± 2	9.7 ± 0.01 (1.12 ± 0.01)	12.9 ± 0.04 (0.84 ± 0.01)	ND	1–50	$Y = 0.0335x + 0.0867$	0.9803	0.3	1.0
2-Octanone	M + D + T	52.8 ± 5.7	9.2 ± 0.03 (1.15 ± 0.01)	9.9 ± 0.03 (1.08 ± 0.01)	12.2 ± 0.03 (0.88 ± 0.01)	3–10	$Y = 0.1335x + 0.0212$	0.9935	0.8	2.5

[a] M = monomer; D = dimer; T = trimer.
[b] dimer only used for quantitation.
ND = not detected.

concluded that the use of SHS-MCC-GC-IMS would provide reliable and consistent data, based on the use of dry and damp fabrics.

3.3. Development of washing protocol using a Tergotometer

An easy and small scale method for simulating the washing process using a Tergotometer was developed. The benefit of using the Tergotometer over a conventional washing machine is the ability to generate and simulate multiple washing processes simultaneously without the need for a major facility with multiple washing machines. In addition, the use of small scale washing conditions has the potential for saving water, electricity, detergents and the washing time. As a first step, a preliminary washing protocol was developed using the Tergotometer.

The present investigation focused on the wash performance for slightly and soiled laundry with tap water classified as slightly hard i.e. 40–60 mg/L Ca (or 100–150 mg/L $CaCO_3$ or 1–1.5 mmol/L $CaCO_3$) [12]. An estimation of the mass of washing powder, required for the Tergotometer, as compared to a domestic washing machine was carried out [13]. The calculated mass of detergent for 2.5 g of garment was approximately 0.05 g. In a domestic washing machine, water consumption is dependent upon several factors including the amount of the load (how many garments are added into the washing machine) and number of cycles (of water). In this study the volume of water was not considered due to the physical limitations of the Tergotometer. Therefore, 0.05 g of washing powder was dissolved in 300 mL of tap water; this homogeneous detergent solution was transferred into one of the reaction vessels of the Tergotometer. The Tergotometer washing process was carried out using the following conditions: a specified temperature, physical agitation using a rotor speed of 200 rpm; and, a washing duration of 10 min. Finally, each garment was then rinsed in clean tap water (500 mL) at a specified temperature for 3 min; and then the rinsing cycle was repeated.

A preliminary investigation was undertaken to investigate the influence on VC loss or retention at two specified temperatures, selected to represent a desirable ambient wash temperature of 20 °C, as a substantive energy saving benefit and a temperature indicative of the average washing temperature in Europe i.e. 50 °C [14].

Sock samples, from two volunteers, were used to investigate the influence of temperature on VC presence/absence in the washing process. The results are shown in Table 3. Two VCs were detectable, namely dimethyl disulfide and dimethyl trisulfide. It is observed that the higher washing temperature (50 °C) is more effective at removing the two VCs; consequently the lowering of the wash temperature resulted in less efficient removal. Due to the interest in the influence of low temperature washing (as a future energy saving measure) all future tests were done at 20 ± 2 °C.

3.4. Sock study

The concentration of VCs (ng/g, fresh weight) was determined pre-wash (dry) and post-wash (damp) for sock sub-samples using SHS-MCC-GC-IMS. Three specific VCs were determined, namely, dimethyl disulfide, dimethyl trisulfide and butyric acid in sock sub-samples. Their summarised results are shown in Fig. 3 (detailed concentrations are in the Supplementary Information, Table S1).

All three volatiles were detected in sock sub-samples from five volunteers (A, D, E, F and H). The highest individual sub-sample concentration identified was for butyric acid (1772 ng/g) from Volunteer E, in the ball section of the sock (Supplementary Information, Table S1). However, in general terms the highest overall concentrations were obtained in the following order: dimethyl disulfide > butyric acid > dimethyl trisulfide. In all cases the washing process considerably reduced the concentration of VCs in socks

Table 3
Influence of washing temperature on VC profile from socks ($n = 1$).

Participant sock code	Dimethyl disulfide (ng/g)						Dimethyl trisulfide (ng/g)					
	Temperature: 20 °C			Temperature: 50 °C			Temperature: 20 °C			Temperature: 50 °C		
	Pre-wash (dry)	Post-wash (damp)	% reduction	Pre-wash (dry)	Post-wash (damp)	% reduction	Pre-wash (dry)	Post-wash (damp)	% reduction	Pre-wash (dry)	Post-wash (damp)	% reduction
A	1129	173	85	597	ND	100	10.1	3.8	63	20.3	5.9	71
C	ND	ND	ND	ND	ND	ND	12.7	4.3	66	24.2	6.6	73

ND = not detected.

from the pre-wash (dry) to the post-wash (damp) stage with average % reductions in concentrations ranging from 32–79% for dimethyl disulfide; 74–93% for dimethyl trisulfide; and, 58–93% for butyric acid. These significant overall reductions in the three VCs during the course of the washing process illustrate the effectiveness at 20 °C with the non-perfumed washing powder. In the preliminary washing study, the developed washing protocol at low temperature (20 °C) was successfully employed to remove the VCs from the sock samples.

3.5. T-shirt study

The underarm region has been acknowledged as a key contributor to the generation of human body odour. The underarm is the place which has the high density of skin glands, together with the widest diversity of microorganism species [15–17]. Various compounds are secreted from these glands that interact with skin microorganisms and forms the characteristic intense odour, known as under arm or axillary odour. Literature has been reported that the characteristic odour that the underarm produces is due to the wide variety of volatile compounds [18,19] including 3-methyl-2-hexenoic acid [20].

The next stage of this investigation describes the identification and quantification of VCs associated with T-shirts in direct contact with the underarm during physical activity, and the subsequent laundering (washing) and drying processes. Initially, physical and sensory perception of the whole T-shirt was done (Table 1) for each of the 9 participants. A descriptor of the physical appearance of the T-shirt, in terms of its degree of (mal) odour and dampness was made. In addition, an olfactory grading was done of each T-shirt to assess its preliminary malodour post-exercise. One individual (Participant 6) was identified as having a more intensely malodorous T-shirt than the others. It was possible to repeat this malodour grading on the sub-sampled T-shirts *in-situ* within the collection vials pre-wash (dry), post-wash (damp) and post-wash (dry). The sub-sampled T-shirt from each participant and their associated malodour grades are shown in Fig. 4. All samples were assessed by one assessor prior to instrumental analysis. In this experiment in between pre-wash (dry) and post-wash (damp), samples were analysed by SHS-MCC-GC-IMS. The average malodour grading ranged from 4–8, 2–4, and 1–3 for the sub-samples pre-wash (dry), post-wash (damp) and post-wash (dry), respectively. A significant reduction of malodour intensity was recorded between the post-wash (damp) and post-wash (dry) sub-samples (ranging from 25 to 98%), probably as a result of evaporation of malodorous VCs. It is also observed that the malodour grading was reduced, in all cases, between the pre-wash (dry) and post-wash (damp) stages i.e. as a result of the washing process.

SHS-MCC-GC-IMS analysis of the T-shirt sub-samples was done and numerous VCs observed (in chromatograms). However, due to the limitation of the current data base all VCs present in the chromatograms were not identifiable. The significant VCs detected, using our own in-house data base [8] were ammonia, dimethyl trisulfide, 2- heptanone, 2-octanone and 2-nonanone. It was not possible to quantify ammonia due to its high signal resulting in a distorted peak shape under non-ideal separation conditions that has been characteristic of it in this study and previously [8]. The study thus focused on the determination and quantitation of dimethyl trisulfide, 2- heptanone, 2-octanone and 2-nonanone.

To study how well these VCs adhered to the T-shirt sub-samples during the laundry process i.e. pre-wash (dry) to post-wash (damp), as well as the drying process i.e. post-wash (damp) to post-wash (dry), the concentration (ng/g) were determined. The results are shown in Fig. 4 (with full details given in Supplementary Information, Table S2). In many cases T-shirt sub-samples from individual participants where VCs were not detected then these have been omitted from Fig. 4. The ketone VCs i.e. 2- heptanone, 2-octanone and 2-nonanone, which were detected from the male participants have previously been reported in the literature as human body odour compounds [21,22]. The VC dimethyl trisulfide which was also present in the T-shirt profiles of four of the participants (M6-M9) has been previously reported in soiled fabric samples [4]. In addition, the major VC in the odour profile of T-shirt sub-samples was ammonia; this has previously been reported in a study on human body odour detection using ion mobility spectrometry [23].

3.6. Source of malodour compounds

This research has identified six volatile compounds by SHS-MCC-GC-IMS from soiled clothing pre- and post-wash, at low temperature, using a non-perfumed version of a leading granular laundry detergent. The results indicate the persistence of the VCs post-wash (under damp and dry). As a consequence the data presented needs to be considered to determine the importance of the findings in the context of the efficiency of the laundry process. These issues will now be considered.

This study has focused on the axillary region in determining the level of male human odour after physical exercise. The sweat and sebum emanating from the axilla region is odour-free and consists of long-chain fatty acids, from the hydrolysis of triglycerides by skin and bacteria lipases, and a range of other compounds which become odoriferous after being metabolized by aerobic bacteria on the axillary skin surface into a range of acids, alcohols, aldehydes [24–26]. The dominant bacteria that have been identified in the most areas of the axilla region are staphylococci and corynebacteria and propionibacteria and staphylococci in sebaceous areas [27,28]. In addition, micrococci and *Malassezia* spp. have also been identified in the axillae region [16,29] with bacteria population densities varying on an individual basis [30]. Troccaz et al. [28] have reported that the bacterial genera responsible for the conversion of odourless apocrine sweat, from the axillary region, to the 'classic' male locker room smell are *Corynebacterium* spp. and some *Staphylococcus* spp. By using the VCs it is possible to identify and hypothesize on the metabolic pathways that have led to their detection. The presence of the common axilla bacteria (*Corynebacterium* spp. and *Staphylococcus* spp.) produce a range of sulphur-containing VCs. The sulphur-containing VCs can be derived from one of two possible L-methionine catabolism pathways (Fig. 5) [30]. In one

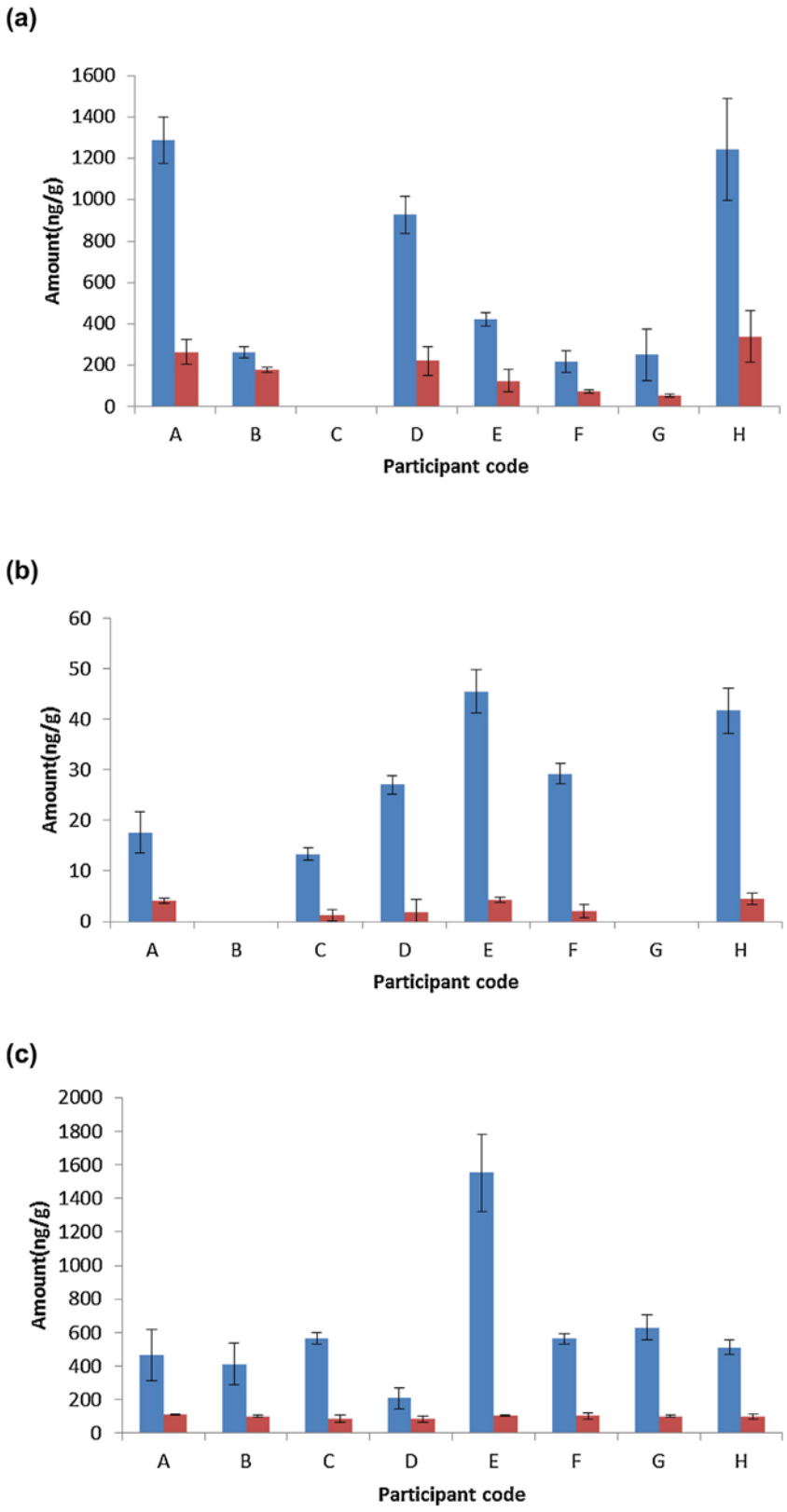

Fig. 3. Amount of three volatile compounds (a): dimethyl disulfide; (b): dimethyl trisulfide; and (c): butyric acid before and after washing in sock samples note: amount of volatiles before wash and amount of volatiles after wash (mean ± SD, $n = 3$).

pathway L-methionine is directly cleaved to form the intermediates α-ketobutyrate, methanethiol and ammonia. Alternatively the L-methionine is converted to the α-keto-γ-methylthiobutyric acid, by transamination, and then subsequent reductive demethiolation to the intermediate products (α-ketobutyrate, methanethiol and ammonia). It has then been postulated that rapid

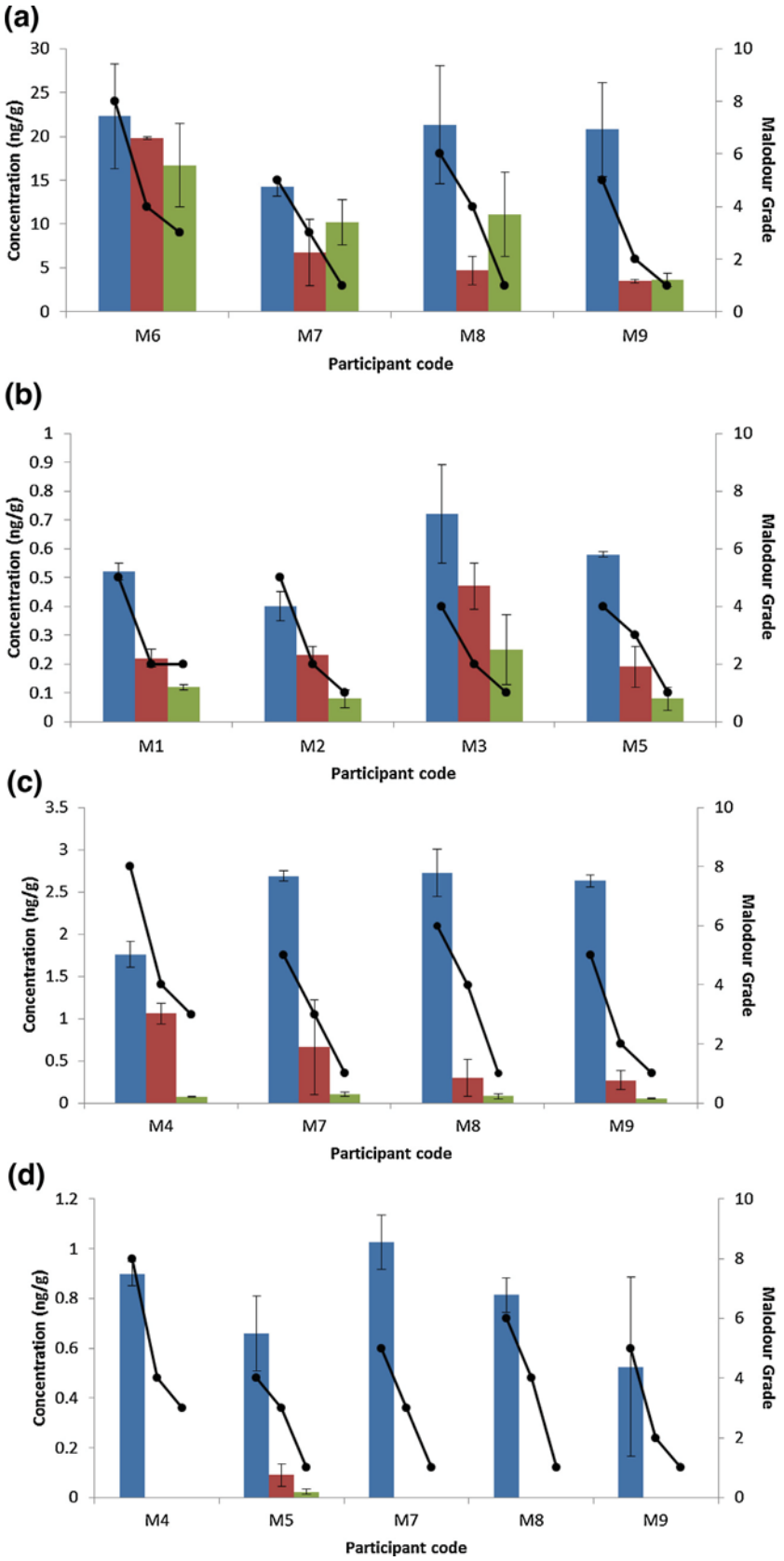

Fig. 4. Amount of four volatile compounds (a): dimethyl trisulfide; (b): 2-heptanone; (c): 2-octanone; and (d): 2-nonanone, and their malodour grades, present in T-shirt samples before and after washing note: amount of volatiles before wash, amount of volatiles after wash damp and amount of volatiles after dry, error bars indicate mean ± standard deviation.

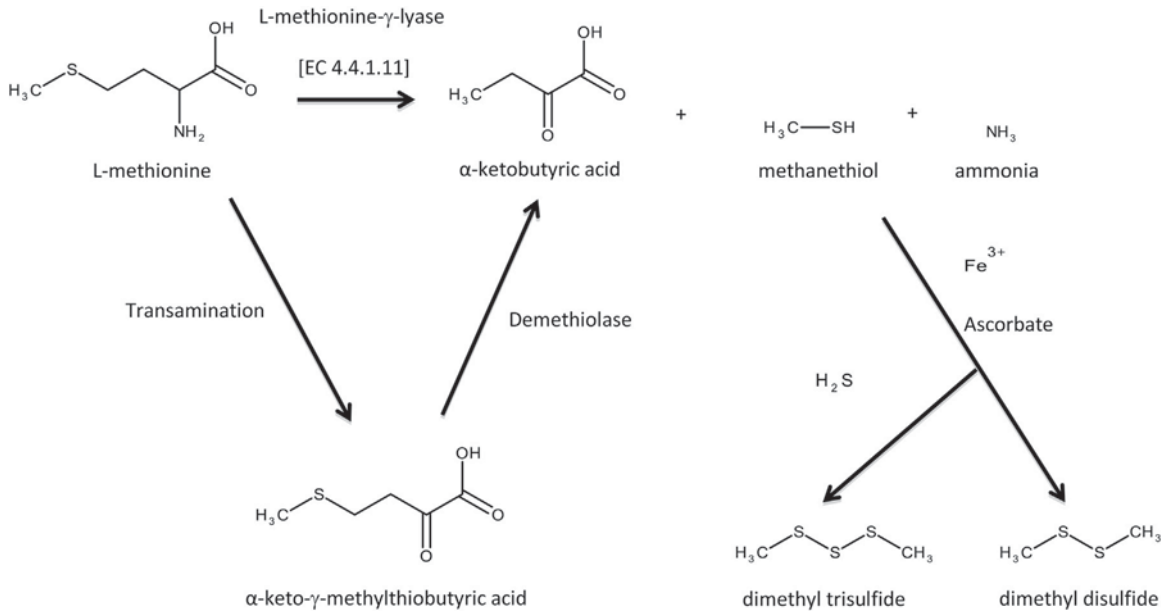

Fig. 5. A biosynthetic pathway for formation of dimethyl disulfide and dimethyl trisulfide from methionine.

autoxidation, mediated by ascorbate and transition-metal ions, will form dimethyl disulfide and dimethyl trisulfide. The quantification of dimethyl disulfide and dimethyl trisulfide, as well as the anecdotal identification of ammonia concurs with this hypothesis. The presence of ammonia has been detected in these samples by MCC0-GC-IMS [8]. The formation of butyric acid has been hypothesized due to a modification of the biosynthetic pathway using acetyl-coenzyme A producing fatty acids with an even number of carbon atoms [30]. Butyric acid has been detected in the headspace of *Staphylococcus* [31] as well as butyric acid and 2-heptanone pre- and post-wash samples from *Staphylococcus epidermidis* inoculated cotton fabric in the presence of triolein, a triglyceride [26]. Finally, the presence of the three ketones can be hypothesized to occur from the further degradation of fatty acids by bacteria. It was reported [30] that the detection of odd carbon number ketones i.e. 2-heptanone and 2-nonanone, that result from the metabolic breakdown of even carbon number fatty acids are quite common whereas it is more rare for even carbon ketones (i.e. 2-octanone) to occur.

If the sources of the VCs can be associated and linked with known bacteria in the axillary region of male volunteers with any certainty then what of their odour. Based on the human olfactory grading data we know that the overall malodour reduces in its intensity post-wash (damp and dry). However, this olfactory grading is both highly subjective and by definition based on the sensory detection of the entire sample at the same time. The use of a chromatographic separation system precludes this, by definition. Never the less it is possible to derive an estimate of the malodour by identifying the odour of each specific compound. The following odours have been noted for each compound: Butyric acid (strong, rancid butter-like odour), dimethyl disulfide (unpleasant, onion-like odour), dimethyl trisulfide (powerful odour), 2-heptanone (banana-like, fruity odour), 2-nonanone (fruity, floral, fatty, herbaceous odour) and 2-octanone (apple-like odour) [32].

However, the VCs were determined directly on soiled garments, pre- and post-wash, from male volunteers after heavy physical activity. It was noted (Figs. 3 and 4) that the detected VCs persist through the entire laundering process under low wash temperature conditions in the presence of a commercially available and leading granular washing powder (non-perfumed) containing lipase. Previous work by Munk et al. [1,7] investigated the presence of VCs from laundry soiled with sweat, at 30 °C washing temperature, on the resultant odour profile determined by GC with olfactory detection and analysis using aroma extract dilution analysis, AEDA [33]. In contrast to our work they collected sweat samples using pre-cleaned swatches by wiping the affected axillary regions of male volunteers after exercises. Another major difference from our work was that the swatches, post-sample collection, were extracted using a more aggressive form of sample extraction i.e. liquid-solid extraction with diethylether for a minimum of 15 h, followed by concentration and evaporation of solvent, prior to re-constitution in minimal solvent. This approach would be able to determine a wider range of VCs due to its more aggressive extraction methodology. In our research we sought to mimic the experience of individuals doing the laundry process, by negating any excessive sample preparation, to measure the inherent olfactory malodour only. Post-collection analysis of laundered swatches detected the presence of dimethyl trisulfide and butyric acid (butanoic acid) along with an additional other 39 compounds (known and unknown) [8]. The presence and absence of lipase, in the washing powder, resulted in no difference in the AEDA analysis of dimethyl trisulfide and butyric acid and which remained modestly low. In contrast in our research we identified considerably fewer compounds. However, our in-house developed database contained 34 VCs many of which were common to those in the work of Munk et al. [1,7]. It was evident from our research that the laundry process was not entirely effective at a washing temperature of 20 °C in the presence of the non-perfumed washing powder in removing VCs characterised as malodourous (Figs. 3 and 4). However, commercially available washing powder does contain perfume added to provide an important self-reassurance that the laundry process has been effective. While further studies are required to investigate the influence of the washing temperature on the laundry process it is evident that the presence and identification of key VCs can provide a guide to its effectiveness. The presence of butyric acid, dimethyl disulfide and dimethyl trisulfide and their associated known odours provide a powerful indicator of malodour in the laundry process.

4. Conclusions

This research has identified six VOCs by SHS-MCC-GC-IMS from soiled clothing pre- and post-wash, at low temperature, using a

non-perfumed version of a leading granular laundry detergent. The results indicate the persistence of the VOCs post-wash (under damp and dry). It was noted that the detected VOCs persist through the entire laundering process under low wash temperature (20 °C) conditions. While further studies are required to investigate the influence of the washing temperature on the laundry process it is evident that the presence and identification of key VOCs can provide a guide to its effectiveness. The presence of butyric acid, dimethyl disulfide and dimethyl trisulfide provide a powerful indicator of malodour in the laundry process.

Acknowledgements

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.chroma.2016.02.037.

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