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Investigations into the initial composition of latent fingerprint lipids by gas chromatography-mass spectrometry

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Abstract

A more comprehensive understanding of the variability of latent fingermark composition is essential to improving current fingermark detection capabilities in an informed manner. Gas chromatography-mass spectrometry was used to examine the composition of the lipid fraction of latent fingermarks collected from a population of over 100 donors. Variations in the appearances of chromatograms from different donors were apparent in the relative peak sizes of compounds including free fatty acids, squalene, cholesterol and wax esters. Principal component analysis was used as an exploratory tool to explore patterns in this variation, but no correlation to donor traits could be discerned. This study also highlights the practical and inherent difficulties in collecting reproducible samples.

Introduction

The interaction that occurs between a latent fingermark deposit and a development reagent is directly dependent upon chemical composition. There are many variables that can contribute to latent fingermark composition, including donor traits, exogenous contaminants and deposition factors. The presence of sebum on the fingertips has significant impact on the mass of the deposited fingermark, as well as the relative proportion of lipids within the residue [1, 2]. It is well established that the increase in sebum production that occurs with the onset of puberty has a dramatic effect on the lipid content of fingermarks deposited by adults compared to young children [3-6]. Significant inter-individual variation has been observed in such studies, and as a result, it has been proposed that other differences in skin surface lipid production related to age, sex, diet, metabolic disorders and skin pathology may impact upon latent fingermark composition such that the analysis of this composition may allow these traits to be inferred [1, 5-7]. There is a need for a more extensive understanding of fingermark chemistry for the further development of latent fingermark detection capabilities [6, 8, 9].

Several studies into fingermark composition have been conducted with the aim to establish whether individual traits may be ascertained from fingermark composition, should a fingermark prove too distorted or otherwise imperfect to allow identification based on the ridge detail [1, 7, 10-12]. To date, gas chromatography-mass spectrometry (GC-MS) is one of the most utilised methods for investigations into the lipid fraction of latent fingermarks [5, 6, 13, 14]. Many of these studies have been of a preliminary nature, and as such have not involved more than a small number (<30) of adult donors [1, 7, 8, 15]. The influence of donor traits is difficult to establish from exploratory investigations, as these small donor populations allow only limited representation of different ages,

sexes, ethnicities and lifestyle factors [1, 12]. There are few investigations that document variation within donor populations that are large enough to provide statistically valid datasets, and that can be considered representative of a general population [5, 6]. The volume of multivariate data generated by large-scale analytical studies requires multivariate statistical analysis in order to derive meaningful information from the dataset [1, 7, 16]. One of the most widely used multivariate statistics methods is principal component analysis (PCA) [17]. PCA simplifies the interpretation of large, complex datasets, such as infrared and ultraviolet-visible spectra or chromatograms of complex mixtures, in an objective and reproducible manner [17-20]. This is achieved by reducing data dimensionality through the transformation of multiple variables from the original datasets into a reduced number of new, orthogonal variables known as principal components (PCs), which can also be used to visualise the distribution of samples [17, 19, 21-23]. Such an approach has been used by Croxton *et al.* to highlight the compositional differences between charged and uncharged fingermarks [1].

We present a series of investigations into the variability of the initial composition of latent fingerprint lipids from a statistically relevant donor population, using gas chromatography-mass spectrometry. Compounds of interest were identified from the most abundant peaks commonly encountered in most samples, in conjunction with major sebum and fingerprint constituents described in the literature. Principal component analysis was performed on this data to assess the influence of intra- and inter-donor variation on fingerprint composition. To this end fingerprint deposits from 5 donors were collected at 2 hour intervals over an 8 hour period and daily at a 2-3 day interval to assess intra donor variability, and once only from 116 donors to assess intra donor variability.

Materials and method

Chemicals

Myristic acid (Aldrich, USA), palmitic acid (Fluka Analytical), sapienic acid (Matreya, USA), palmitoleic acid (Sigma-Aldrich, USA), stearic acid (Aldrich, USA), squalene (Sigma-Aldrich, USA), cholesterol (BDH, UK), myristyl palmitoleate (Nu-Chek Prep, Inc, USA), myristyl palmitate (Nu-Chek Prep, Inc, USA), palmityl palmitate (Nu-Chek Prep, Inc, USA), palmityl palmitoleate (Nu-Chek Prep, Inc, USA), oleyl myristate (Nu-Chek Prep, Inc, USA), stearyl myristate (Nu-Chek Prep, Inc, USA), stearyl palmitoleate (Nu-Chek Prep, Inc, USA), palmityl oleate (Nu-Chek Prep, Inc, USA), stearyl palmitate (Nu-Chek Prep, Inc, USA) and dichloromethane (Macron Chemicals, USA) were used as received. A set of standard solutions of the free fatty acids, squalene, cholesterol and wax esters were prepared

as individual solutions in dichloromethane in the concentration range of 0.1 – 50 ppm. All standard solutions were stored at -20 °C before and after analysis to prevent degradation and solvent evaporation.

Sample collection and storage

Fingerprint samples were collected on filter paper circles (25 mm qualitative filter paper, Grade 1; Whatman, UK). Donors were instructed to briefly rub the tips of their middle three fingers on their forehead or nose, and then press each fingertip gently to a filter paper circle for approximately ten seconds. Some donors were required to provide samples using a modified procedure where fingerprints from both hands were deposited sequentially to collect two fingerprints on each filter paper. After the donor removed their hand, the filter papers were wrapped in aluminium foil and labelled with an alphanumeric code. Donors were also asked to fill out a brief survey regarding their age, sex and substances they had recently handled. Samples were analysed within an hour of deposition, or were stored in screw-top jars and transferred to a -20 °C freezer until analysis. Samples collected at locations remote to the laboratory were stored in an ice box until they had been transported to either the laboratory or the freezer.

Sample preparation

Extraction of fingerprint residue from the filter papers was performed in 1.75 mL glass screw-top vials (Thermo Fisher Scientific, Australia) that had been cleaned by rinsing with dichloromethane. Samples that had been stored at -20 °C were allowed to equilibrate to ambient temperature before extraction. Samples were immersed in 750 µL dichloromethane for 2 minutes, with gentle manual agitation to ensure that the filter papers were completely submerged in the solvent. After 2 minutes, the filter papers were removed and discarded, and the sample extracts were then transferred to 2 mL glass crimp top vials (Agilent Technologies, USA). The vials were sealed with aluminium crimp tops (Agilent Technologies, USA), after covering the vial opening with aluminium foil to prevent solvent extraction of plasticisers from the septa, and analysed by GC-MS. Analytical blanks consisting of clean filter papers were prepared and analysed with each set of samples.

Chemical analysis

Chromatographic analysis was performed on a Hewlett Packard 6890 series GC coupled with a Hewlett Packard 5973 mass selective detector (MSD), a 6890N series GC coupled with an Agilent 5973N MSD, a Hewlett Packard 6890A GC coupled with a Hewlett Packard 5973A MSD, a 6890 series GC coupled with an Agilent 5975 inert MSD, and an Agilent 7890A GC coupled with a Agilent 5975C inert XL EI/CI MSD. Full instrumental conditions are described in Table 1.

Table 1: Instrumental conditions for GC-MS

	Gas chromatograph	Column type	Injector	Injection volume	Mass spectrometer
C16:1 isomer comparison	Agilent 7890A	Agilent Technologies HP-Innowax (30 m x 0.25 mm ID x 0.25 μ m d_f)	Agilent 7683B series	1 μ L	Agilent 5975C inert XL EI/CI MSD
Intra-donor variation (1 day)	Hewlett Packard 6890A	Agilent J&W DB-5MS (60 m x 0.25mm ID x 0.25 μ m d_f)	Hewlett Packard 6890 series injector		Hewlett Packard 5973A
Intra-donor variation (1 month)	6890 series	Phenomenex ZB-5MS (30 m x 0.25 mm ID x 1 μ m d_f)	Gerstel MPS2 autosampler		Agilent 5975 inert mass selective detector
Inter-donor variation					

For all sample analysis, the GC oven was programmed from 40 °C, held for 1 minute, then increased from 40 °C to 320 °C at 20 °C/min and held for 30 minutes. The inlet was operated at 320 °C in splitless mode. Helium was used as the carrier gas, at a constant flow rate of 1.1 mL/min. Typical MSD conditions were: solvent delay, 5 minutes; ionisation energy, 70 eV; source temperature, 230 °C; and electron multiplier voltage, 1505.9 V.

For C16:1 isomer comparisons, the GC oven was programmed a) from 40 °C, held for 1 minute, then increased from 40 °C to 260 °C at 10 °C/min and held for 35 minutes; b) from 40 °C to 150 °C at 10 °C/min and held for 50 minutes, then increased from 150 °C to 260 °C at 10 °C/min and held for 5 minutes; and c) from 40 °C to 180 °C at 10 °C/min and held for 50 minutes, then increased from 180 °C to 260 °C at 10 °C/min and held for 5 minutes. The inlet was operated at 270 °C in splitless mode. Helium was used as the carrier gas, at a constant flow of 1.1 mL/min. Typical MSD conditions were:

solvent delay, 3 minutes; ionisation energy, 70 eV; source temperature, 230 °C; and electron multiplier voltage, 2553 V.

Data analysis

The data was pre-processed using Chemstation Data Analysis (Agilent Technologies, USA) through background subtraction of all chromatograms, followed by manual integration of selected peaks (discussed below). Where appropriate, peaks were identified using standards, comparison with the MS library (NIST), or examination of the mass spectra. Replicates from each donor were treated as individual samples in the data matrix. Peak areas were normalised to the sum using Microsoft Excel. Principal component analysis (PCA) of the data was performed using the Unscrambler® X 10.3 software (CAMO Software AS, Oslo, Norway).

Results and discussion

Method development

Sample preparation was based on the methods described by Asano *et al.*, Koenig *et al.* and Weyermann *et al.* [10, 15, 24]. These approaches enable the detection of up to almost 100 lipid compounds, representing most major lipid classes present in sebum [7], while avoiding time-consuming and costly derivatisation procedures [25]. Due to the small amount of material that comprises latent fingerprints, a concentration step is often employed in GC-MS studies, whereby most or all of the extracting solvent is evaporated under nitrogen and the sample is reconstituted to a small volume, before being introduced into the GC [5, 8, 10, 15, 24]. Such a step was not employed in the method used in this study as the evaporation of dichloromethane risks introducing contaminants in the form of water, or plasticisers from the apparatus used to deliver nitrogen gas. Additionally, the small final volume of such pre-concentrated samples (20 – 100 µL) was regarded as a disadvantage, considering the volatile nature of the solvent (dichloromethane), and that large numbers of samples were to be analysed over periods of up to 24 hours.

A relative quantification approach was chosen over absolute quantification to overcome variation in the amount of residue deposited by donors due to differences in fingerprint size or deposition technique. There is no correlation regarding the contribution of lipid material to total fingerprint mass [26], and quantitative differences may be attributed to fingerprint surface area rather than donor traits [1, 24]. Additionally, the amount of lipid can vary considerably, depending on how recently an individual has washed their hands, and if they have replenished the lipid material on their fingertips by touching their face or scalp [27]. In the authors' experience [28-30], donors often

do not reproducibly deposit impressions of the entire fingerprint pattern, which may contribute to intra-donor variation.

Fifteen components including free fatty acids, squalene, cholesterol and some wax esters were identified as the abundant peaks common to most fingerprint samples (further details provided below). The peak areas of each compound were normalised against the sum to determine the relative quantities of each compound in the samples. Some latent fingerprint studies have used internal standards to observe relative changes in composition with time [5, 8, 15] or have determined variation based on peak area ratios to squalene [10]. Normalisation to the sum has been demonstrated to reduce intra- and inter-sample variability compared to normalising to an internal standard [7].

Compound identification

Endogenous lipids

A range of endogenous lipid compounds reported in previous fingerprint studies [1, 6, 8, 10, 15, 24] were identified in fingerprint samples in these investigations. A sample chromatogram of a latent fingerprint is shown in Figure 1. Identification of lipid compounds was carried out using several methods, including comparison with standard solutions, visual comparison with the MS library, or examination of the mass spectra for diagnostic fragment ions. In most chromatograms, squalene formed the largest peak, or was the largest peak attributable to endogenous lipid content. Even-chain saturated and monounsaturated free fatty acids of 12 – 18 carbon units, cholesterol and wax esters were also readily detected in most samples. Palmitic acid and hexadecenoic acid usually comprised the most abundant endogenous compounds after squalene. Pentadecanoic acid was the only abundant odd-chain fatty acid, and often the only one detected in most samples. These observations are consistent with reported literature [5, 6, 15, 24, 26].

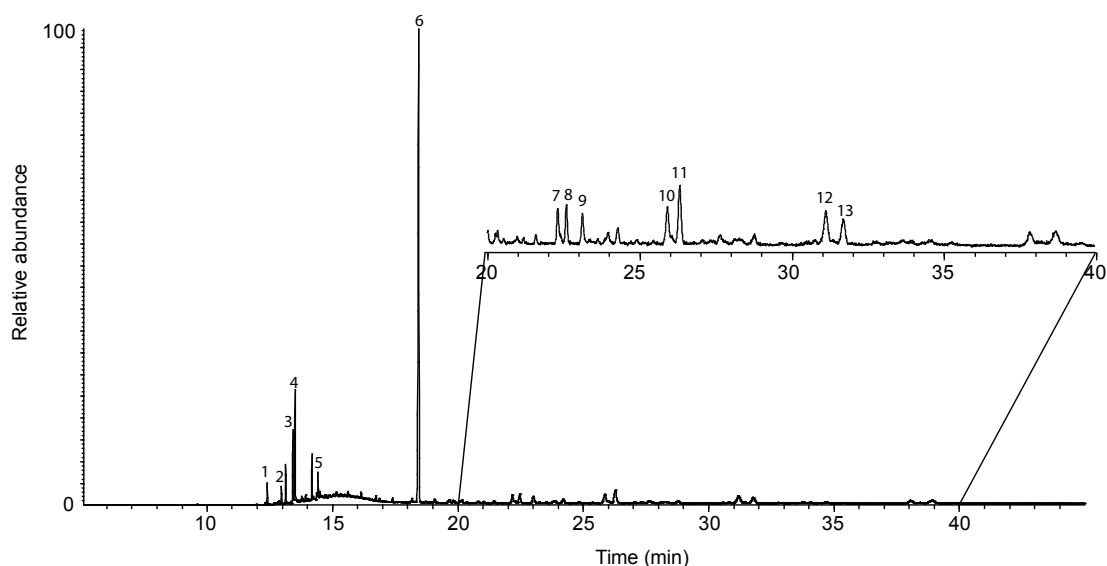


Figure 1: Sample total ion chromatogram (TIC) of a latent fingerprint, showing identified peaks 1. Myristic acid, 2. Pentadecanoic acid, 3. Hexadecenoic acid, 4. Palmitic acid, 5. Oleic acid, 6. Squalene, 7. C30:1 wax esters, 8. C30:0 wax esters, 9. Cholesterol, 10. C32:1 wax esters, 11. C32:0 wax esters, 12. C34:1 wax esters, 13. C34:0 wax esters

Several peaks eluting later in the chromatogram were tentatively identified as wax esters based on comparisons with the MS library; however, such matches were frequently inconclusive or ambiguous. This, coupled with the broad appearance of the peaks [31], indicated the co-elution of isomeric esters, i.e. those containing the same total number of carbon units and double bonds but with varying fatty acids and fatty alcohol species. Co-elution of wax ester isomers is a commonly encountered phenomenon in chromatographic studies of lipid mixtures due to chain length [31-34]. The co-elution of isomeric wax esters was confirmed from a combined examination of the mass spectral data and retention time comparisons with reference compounds.

Table 2: Compounds identified in chromatograms of fingerprint samples with compounds used in PCA noted in bold

Compound(s)	Identification
Dodecanoic (lauric) acid (C12:0)	MS library comparison
Tridecanoic acid (C13:0)	MS library comparison
Tetradecenoic acid (C14:1)	MS library comparison
Tetradecanoic (myristic) acid (C14:0)	MS library comparison, standard
Pentadecenoic acid (C15:1)	MS library comparison
Pentadecanoic acid (C15:0)	MS library comparison
Hexadecenoic acid (C16:1)	MS library comparison, standards
Hexadecanoic (palmitic) acid (C16:0)	MS library comparison, standard
Heptadecanoic acid (C17:0)	MS library comparison
Octadecenoic (oleic) acid (C18:1)	MS library comparison
Octadecanoic (stearic) acid (C18:0)	MS library comparison, standard
Squalene	MS library comparison, standard
Wax esters (C28:0) Myristyl myristate (14:0-14:0) Lauryl palmitate (12:0-16:0) Stearyl decanoate (18:0-10:0)	MS library comparison, examination of MS Examination of MS MS library comparison, examination of MS
Wax esters (C30:1) Myristyl hexadecenoate (14:0-16:1)	Examination of MS
Wax esters (C30:0) Palmityl myristate (16:0-14:0) Myristyl palmitate (14:0-16:0) Stearyl laurate (18:0-12:0) Lauryl stearate (12:0-18:0) Decyl eicosanoate	MS library comparison, examination of MS, standard Examination of MS, standard Examination of MS Examination of MS Examination of MS
Cholesterol	MS library comparison, standard
Wax esters (C32:1) Palmityl hexadecenoate (16:0-16:1) Myristyl oleate (14:0-18:1) Oleyl myristate (18:1-14:0)	Examination of MS Examination of MS Standard
Wax esters (C32:0) Palmityl palmitate (16:0-16:0) Stearyl myristate (18:0-14:0) Myristyl stearate (14:0-18:0) Lauryl eicosanoate (12:0-20:0)	MS library comparison, examination of MS, standard Examination of MS, standard MS library comparison Examination of MS
Wax esters (C34:1) Stearyl hexadecenoate (18:0-16:1) Palmityl oleate (16:0-18:1)	Examination of MS Examination of MS, standard
Wax esters (C34:0) Stearyl palmitate (18:0-16:0) Palmityl stearate (16:0-18:0) Arachidyl myristate (20:0-14:0)	MS library comparison, examination of MS, standard Examination of MS Examination of MS

The structures of the wax esters were determined by examination of the mass spectra of each peak. The total chain lengths of the wax esters and the presence of double bonds were established from the mass of the molecular ion, while the molecular structures were determined through identification of diagnostic fragment ions corresponding to constituent fatty acids and alcohols [31, 34-36]. The presence of two or more fatty acids or fatty alcohols in the same peak therefore indicated that most peaks with longer retention times consisted of several isomeric wax esters.

Some wax ester standards were found to have a slightly longer retention time than the corresponding fingerprint component. Interestingly, this disparity was only seen in unsaturated wax ester standards that contained palmitoleic acid, despite mass spectral data indicating the presence of hexadecenoic acid in the sample peaks. The retention times of other monounsaturated wax ester standards that contained oleic acid or an unsaturated fatty alcohol matched those of the corresponding sample peaks.

It is unclear as to why the palmitoleic acid esters might have eluted separately from the sample peaks. A possible explanation is that the wax esters in the sample extracts are branched-chain isomers of the straight-chain standards, as branch-chain esters are often eluted sooner than straight-chain isomers [31, 33], though this seems unlikely due to the isomeric standards matching the retention times of the sample peaks. Additionally, Fitzgerald *et al.* report that human wax esters are predominantly straight chain, saturated structures, though they do include some branched isomers [31]. Whether branched-chain wax esters are present in detectable quantities in the sample extracts is difficult to confirm, as the position of methyl branches cannot be determined from analysis of intact esters [37]. Another possibility is that the monounsaturated wax esters are positional isomers of the standards, which can affect retention times [38, 39], but again, this does not explain the matching retention times of the other unsaturated wax ester standards. Further investigations are required to fully explore the identities and structural isomers of the wax esters present in fingerprints.

A characteristic feature of human sebum is the prevalence of the $\Delta 6$ pattern of unsaturation [34, 40-44]. The majority of unsaturated free fatty acids, wax esters and sterol esters produced by adult human sebaceous glands exhibit an unusual desaturation position of $\Delta 6$ rather than the more typical $\Delta 9$ pattern [40, 44-46]. No studies of fingerprint composition have reported the detection of sapienic acid (C16:1 $\Delta 6$), while its isomer palmitoleic acid (C16:1 $\Delta 9$) is named as one of the most abundant monounsaturated fatty acids in fingerprint residue [6, 8, 10, 25]. Several studies have reported the detection of wax esters containing palmitoleic acid or palmitoleyl alcohol in latent fingerprints [10, 14, 15]. Conversely, in dermatological research, while sapienic acid is often

mentioned as a major component of sebaceous free fatty acids, palmitoleic acid is not [34]. Work by Pappas *et al.* found that exogenously applied, ³H labelled palmitoleic acid was not incorporated into wax esters except as extension products [47].

In light of this, standard solutions of sapienic acid and palmitoleic acid were analysed to determine whether one or both species were present in latent fingermarks. The two isomers exhibited identical retention times under the GC-MS conditions used for fingermark analysis, and comparison of the mass spectra found that the standards were also isobaric, i.e. exhibited identical fragmentation patterns. A column with a highly polar stationary phase was subsequently utilised, using a variety of isothermal temperature programs; however, resolution of the two fatty acids still could not be achieved. This behaviour may account for the successful use of palmitoleic acid (and palmitoleate wax esters) as reference standards in fingermark studies [1, 15, 48]. Positional isomers of fatty acids can be difficult to separate using GC if the difference in bond position is not a large one, and cannot be distinguished based on mass spectra unless tandem mass spectrometry is employed [39]. As the identity of the monounsaturated C16 fatty acid could not be confirmed, it is referred to throughout this paper by the generic name hexadecenoic acid.

The range of detected compounds, namely fatty acids and wax esters, was smaller than those reported in similar studies [7, 15]. This can be attributed to the lower concentrations of the sample extracts. It should be noted that the purpose of these investigations was not to characterise or quantify the components of fingermark residue, but to identify the most abundant common species to be utilised in the construction of PCA models. Based on chromatograms obtained from 10 donors, fifteen components were selected (noted in bold in Table 5.2). While these components were common in samples to most donors, not all fifteen were present in all samples in detectable quantities, particularly the wax esters, cholesterol and stearic acid. The inclusion of these compounds was justified on the basis of the greater inter-donor discrimination that would be possible compared to only utilising those common to samples from all donors [7].

Exogenous contaminants

Donors were not asked to wash their hands prior to sample collection in order to obtain samples that may be considered more realistic than those deposited following any sort of cleaning as part of the collection protocol. The presence of exogenous contaminants from the hands is expected to be commonly encountered in 'real' fingermarks, and the analysis of samples consisting only of endogenous skin secretions will have limited value beyond theoretical interest. Free fatty acids, squalene, cholesterol and wax esters are all common ingredients of skin products [8, 33, 36], and

therefore the presence of such in fingermark samples may have an effect on classification compared to 'clean' samples.

In addition to the lipid compounds described above, chromatograms of samples from donors who had used skin products were often complicated with additional major peaks (Figure 2). The identities of many of these compounds could not be determined from comparison with the MS library. Those that were, such as isopropyl myristate, are believed to be sourced from skin products, being common ingredients of such [49]. These peaks often complicated peak integration for data processing, as analytes of interest were co-eluted or incompletely resolved. Cholesterol, for example, was sometimes incompletely resolved from a compound identified by database comparison as vitamin E acetate, used as an antioxidant in skin products. Vitamin E acetate is a common component of skin lotions and moisturisers [49], and was the most frequently encountered identified contaminant in the fingermarks of donors who used such products. Samples from 21 (4 male, 17 female) of the 116 donors contained this compound. Additional peaks, identified as long-chain alkanes, were presumed to originate from the substrate. It has been demonstrated that a number of organic contaminants, including long-chain alkanes, may be derived from paper-based sources [50]. The presence, though not the identity, of contaminants in fingermark samples derived from extraction from porous substrates has been previously reported [15]. There is a further possibility that at least some of these alkanes may in fact be fingermark components, as alkanes and other hydrocarbons are minor constituents of human sebum [51]. Attempts to pre-clean porous substrates by sonication for 15 minutes in dichloromethane were unsuccessful in completely removing these compounds.

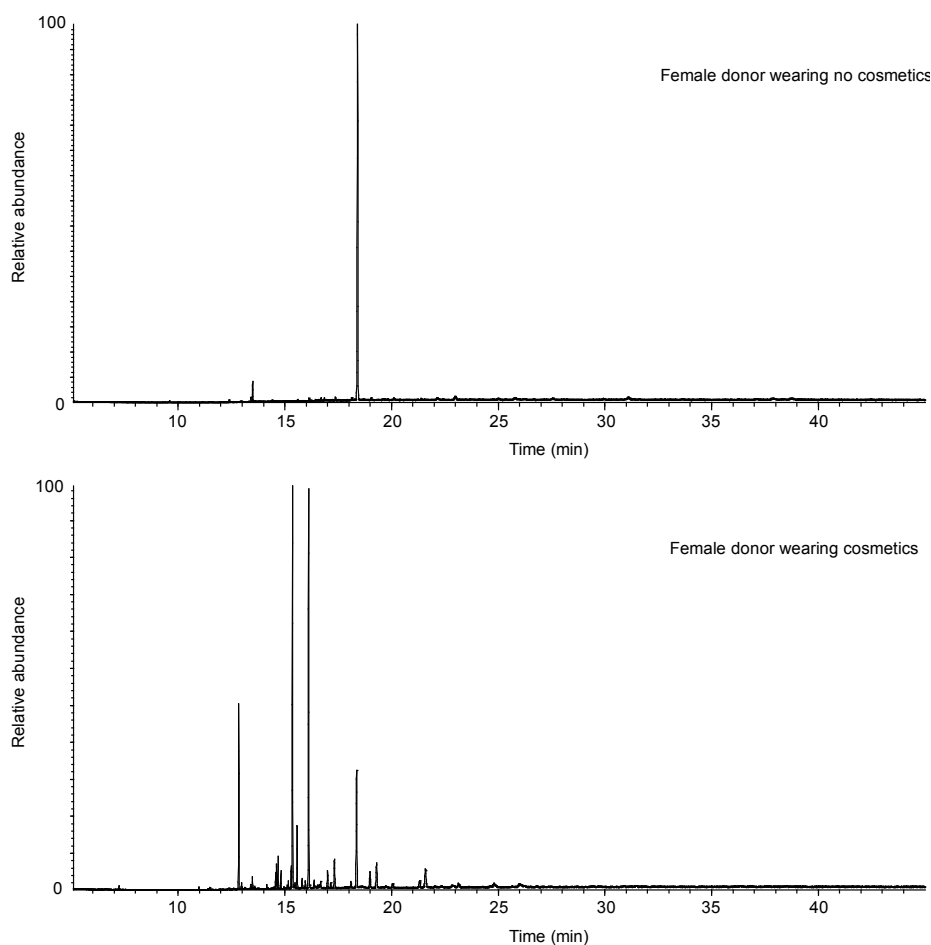


Figure 2: Example of exogenous peaks introduced into TICs of samples from two adult female donors by use of cosmetic products

Intra-donor variation

Investigations into intra-donor variation over short- and long-term periods are necessary to ascertain whether or not donor classification could be affected by natural changes in lipid composition over time. The determination of such variation is crucial to method validation [7, 8, 10, 15, 24]. If an individual's fingerprint composition was shown to vary significantly over time, and this variance was found to be as great, or greater, than that observed between different individuals, using fingerprint composition as a means to infer individual characteristics or estimate fingerprint age could not be considered a viable approach [52].

Variation over one day

It has been demonstrated that variation in skin surface lipid composition may occur over periods of several weeks, but it is unclear as to whether there is any significant short-term variation [53, 54]. While the secretion rate of the sebaceous glands of the forehead demonstrates a circadian rhythm, with maximal rates around midday, to the best of the authors' knowledge, it is not known if this is

reflected in the relative concentrations of individual constituents [55-57]. To investigate whether fingerprint lipid composition was affected by time of day, samples were collected in triplicate from five donors (Table 3) every two hours from 9:00 am – 5:00 pm, providing a total of 15 samples per donor. Samples were collected from the middle three fingers of the same hand each time. Samples were collected from each donor on separate days over a two week period. At each sampling time, donors were asked to fill in a short survey regarding any recent activities that may affect the quantity and/or quality of substances present either on their face or hands, including the handling of possible contaminants such as food or other substances.

Table 3: Summary of donor information

Sex	<i>n</i>	Age (years)	<i>n</i>	Recent skin product use	<i>n</i>
Female	3	20 – 29	3	Yes	4
Male	2	30 – 39	2	No	1

No general trends related to changes in sample composition as a function of time of day were identified from the appearance of the chromatograms. The relative amounts of free fatty acids exhibited variation between samples from the same donor, including replicate samples from the same sampling time, as well as between samples collected at different times throughout the day. Significant inter-donor variation was evident in the visual appearance of the chromatograms. The relative peak heights of wax esters and free fatty acids, particularly hexadecenoic and palmitic acid, appeared to vary between donors, such that samples from some donors could be easily differentiated by these characteristic features. Notably, one donor (DB012) reported using cosmetic products and also regularly applied vitamin E enriched cocoa butter to their hands throughout the day. The presence of vitamin E acetate and other additional peaks in chromatograms from this donor was attributed to these products.

PCA performed on the dataset (75 chromatograms) and examination of the resultant Scree plot revealed that 99.23 % of the total variance of the dataset was accounted for in the first 3 PCs. The scores plot generated from the first 3 PCs (Figure 3) revealed that while the dataset comprised distinct groupings, samples from each donor in the dataset formed loose clusters, indicating greater inter- than intra-donor variation. While PC3 only accounts for 0.58 % of the total variance within the dataset, it provided additional discrimination by separating samples from donors CA006 and CB007.

Clusters from donors DB012 and CB003 were projected very close together, making visual discrimination of these groups difficult. Additionally, samples from some donors appeared to form more cohesive clusters than other, suggesting differences in the extent of intra-donor variation between donors, as a function of direct compositional variation or sample reproducibility.

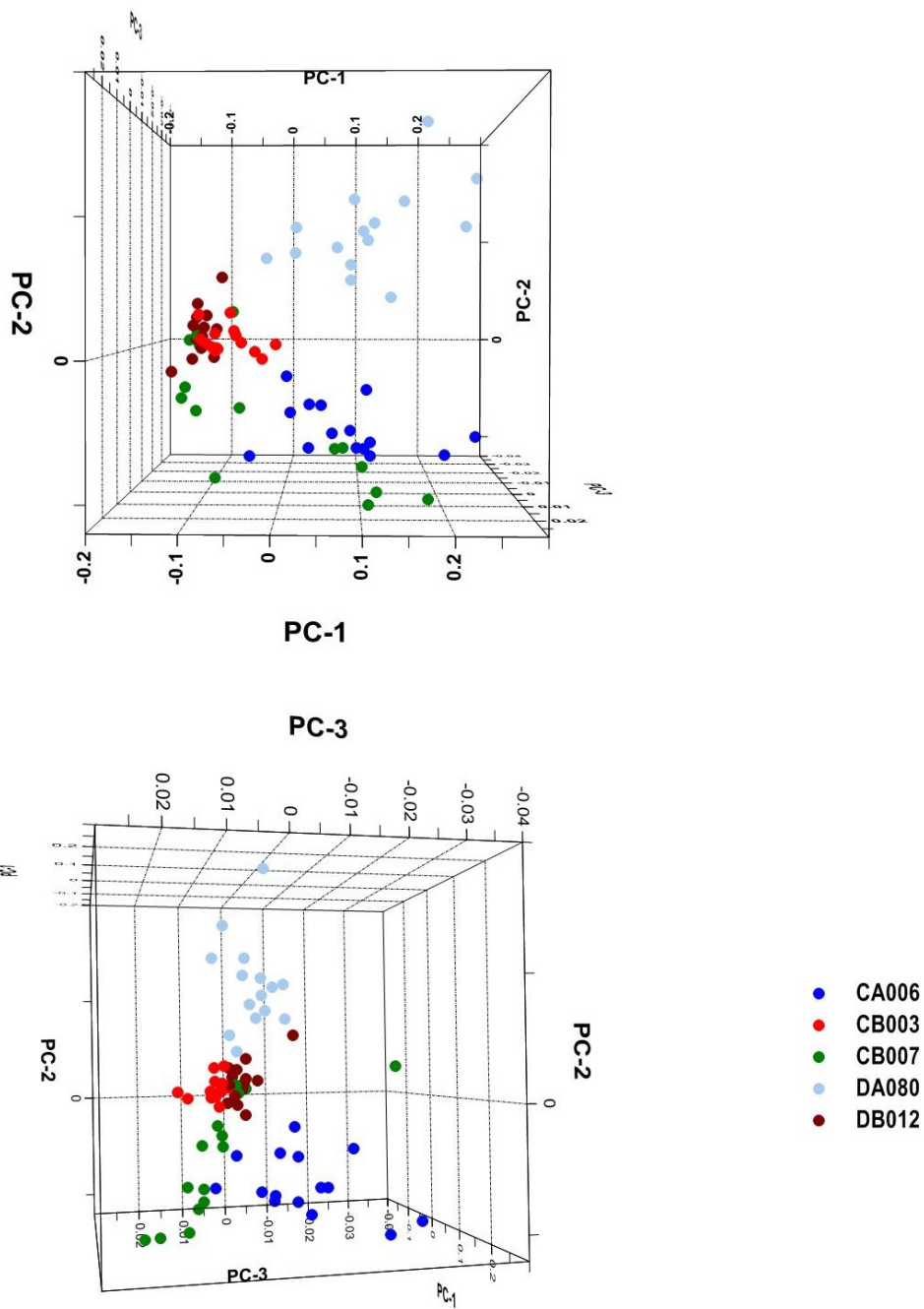


Figure 3: 3-dimensional scores plot generated from the first 3 PCs, from two perspectives, demonstrating the distribution of fingerprints collected from five donors over the course of eight hours

For most donors, replicate samples from the same sampling time were scattered throughout each cluster, indicating that there was no clear trend in fingerprint composition over an 8 hour sampling period, and as much variation in lipid composition between replicates as between samples collected at different times. Sebum accumulates on the skin surface over the course of the day unless it is removed by washing, so it is possible that changes in secretion composition would be masked by dilution in the accumulated lipid already present on the surface of the skin. It is important to note that samples from one donor (CB007) were projected with a distinct separation between samples collected at 9:00 am – 1:00 pm, and samples collected at 3:00 – 5:00 pm. This donor did not report handling any food or other substances between these sampling periods, and so this change is unlikely to be due to exogenous contamination. The two clusters were separated primarily along PC3. From examination of the chromatograms, it was noted that samples from this donor typically contained very few free fatty acids and wax esters in detectable levels, but the relative areas of these peaks increased in samples collected in the afternoon. The gradual accumulation of sebum on the skin surface may account for fingerprints sampled later in the day containing larger amounts of these components than samples collected in the morning and early afternoon.

The factor loadings for the first 3 PCs were utilised to identify the compounds that contributed to the differentiation of samples within the scores plot (Figure 4). The loadings plot for PC1 revealed significant negative correlation to squalene, therefore projection of samples along PC1 is based primarily upon the relative abundance of squalene in fingerprints. Samples which contain relatively large abundances of squalene attain negative scores on PC1, while samples with low relative amounts of this compound have positive scores on PC1. The abundance of squalene, the most abundant individual species in sebum, is considered to be directly correlated to sebaceous gland activity [32, 58]. The loadings plot for PC2 revealed significant negative correlation to palmitic acid, as well as negative correlation to squalene, and some positive correlation to several of the wax esters. The loadings plot for PC3 revealed significant negative correlation to hexadecenoic acid and oleic acid, as well as significant positive correlation to palmitic acid, and some positive correlation to squalene and several wax esters. The relative amounts of free fatty acids in sebum are thought to be indicative of bacterial activity on the skin surface in hydrolysing sebaceous triglycerides [15, 51, 54, 59, 60]. Discrimination between samples therefore arises primarily from differences in relative amounts of the most abundant lipid compounds – squalene and long-chain free fatty acids [6, 8, 15].

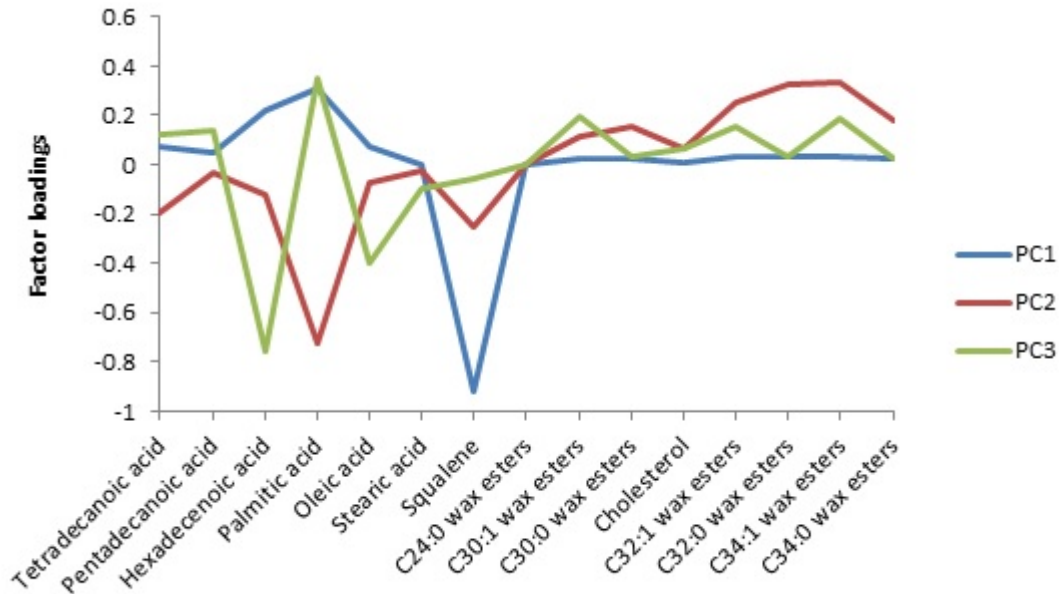


Figure 4: Factor loadings for the first 3 PCs

Several investigations have concluded that although there was some variation in the concentrations of certain fingerprint lipids over the course of a day, this variation was not statistically significant [8, 10, 12]. Whether intra-donor variation occurs to such an extent to impact upon inter-donor discrimination is difficult to elucidate from these studies, as typically only one donor was monitored in each case [8, 12, 15, 24]. The study reported here shows that this is not a reliable approach, as the extent of variability can be markedly different between donors. Guidelines recently proposed by the International Fingerprint Research Group recommend that proof-of-concept evaluations of novel fingerprint development methods utilise fingerprints from at least 3 – 5 donors [61]. This and other such recommendations may also benefit analytical studies of fingerprint composition in demonstrating compositional variation.

Variation over one month

Similar to the studies discussed above, previous reports of intra-donor variation of fingerprint composition over periods of several days to weeks have typically monitored one donor, or have only sampled at infrequent intervals [6, 15, 24]. Samples were collected in triplicate every 2 – 3 days over the course of 29 days from four of the donors who had participated in the short-term intra-donor variation study. 36 – 39 samples were collected in total from each donor, as two donors were not available for sample collection on two separate days due to illness. Sample collection was carried out during the morning, at the convenience of the donors.

PCA performed on the dataset (150 chromatograms) showed that 99.26 % of the total variance of the dataset was accounted for in the first 3 PCs. The scores plot generated using these PCs (Figure 5) was largely similar in appearance to Figure 3 in the relative positioning of samples from each donor, with most samples from the same donor projected as broad groupings. This suggests that any variation in intra-donor composition that may have occurred over the 29 day period was not significant enough to affect visual discrimination between donors in this very small population.

Samples from donor CA006 formed two separate groups, separated primarily along PC3. Replicate samples from the same day were present in both groups, discounting the possibility of a sudden, marked change in fingermark composition during the sampling period. This highlights a major problem frequently encountered in latent fingermark analysis: the obtaining of reproducible samples [6, 8, 15]. In a research context, reproducible fingermark deposition would require strict control over parameters such as cleaning of donors' hands before sample collection, length of contact with the substrate, pressure of fingertips, etc. Such measures have been explored [15, 62], but doing so risks divorcing the experimental approach from the 'reality' of incidental fingermark deposition. Aside from issues concerning sample homogeneity, there did not appear to be any significant trends related to compositional differences from samples obtained from individual donors over a time period of at least several weeks.

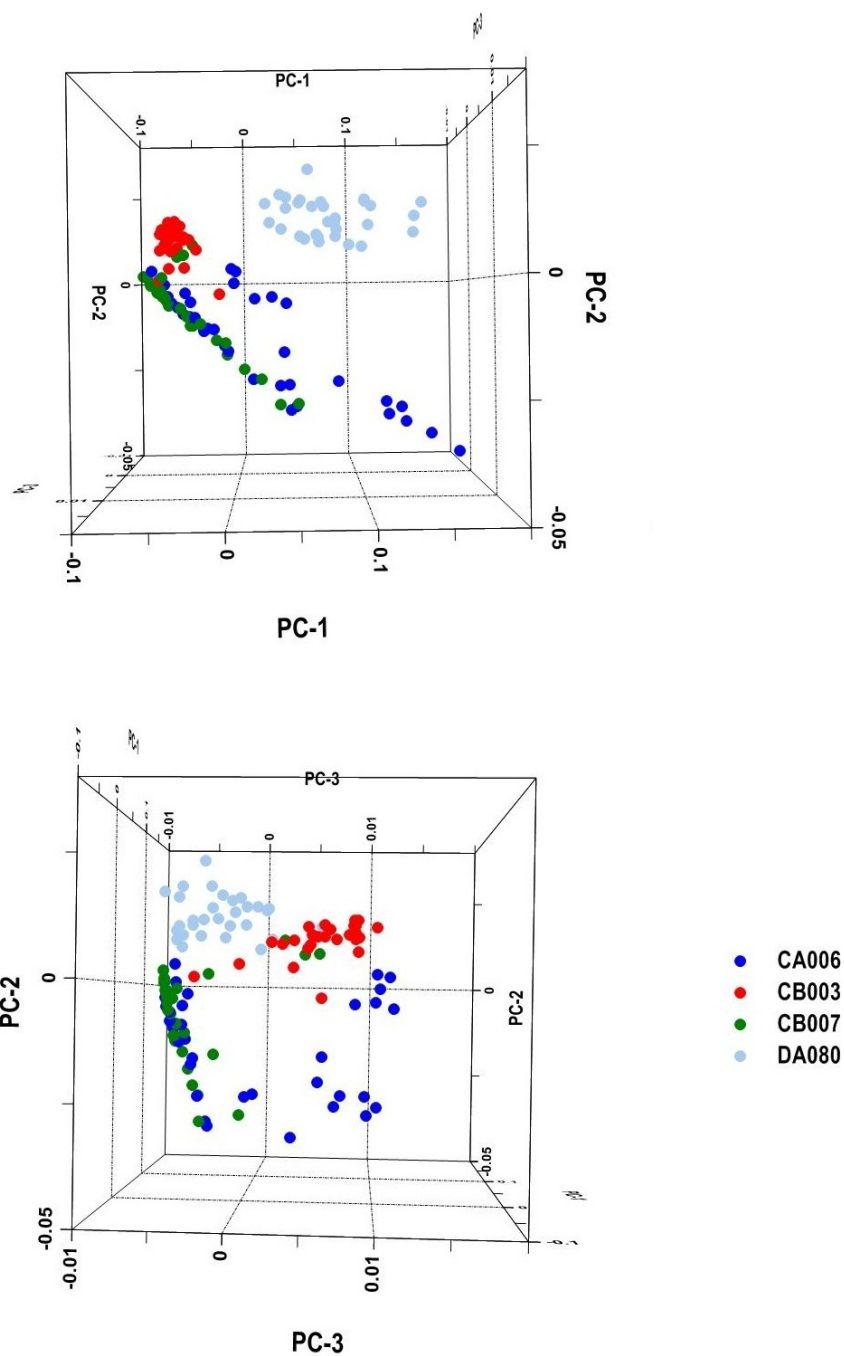


Figure 5: 3-dimensional scores plot generated from the first 3 PCs, from two perspectives, demonstrating the distribution of fingermarks collected from four donors over 29 days

The factor loadings for the first 3 PCs were utilised to identify the compounds that contributed to the variance within the dataset (Figure 6). The loadings plots for the first two PCs were almost identical to those in Figure 4. The loadings for PC1 revealed significant negative correlation to squalene, as well as some positive correlation to palmitic acid and hexadecenoic acid. PC2 exhibited

significant negative correlation to palmitic acid, and some positive correlation to several wax esters. The loadings for PC3 revealed significant positive and negative correlation to various wax esters. As the third PC is influenced here by wax esters, rather than free fatty acids, samples from donors CB007 and CA006 are no longer resolved as well along this PC. The differences in the factor loadings of the third PCs in this and the above sections can be accounted for by the absence of donor DB012 from the former sample population, as well as the difference in size between the two datasets.

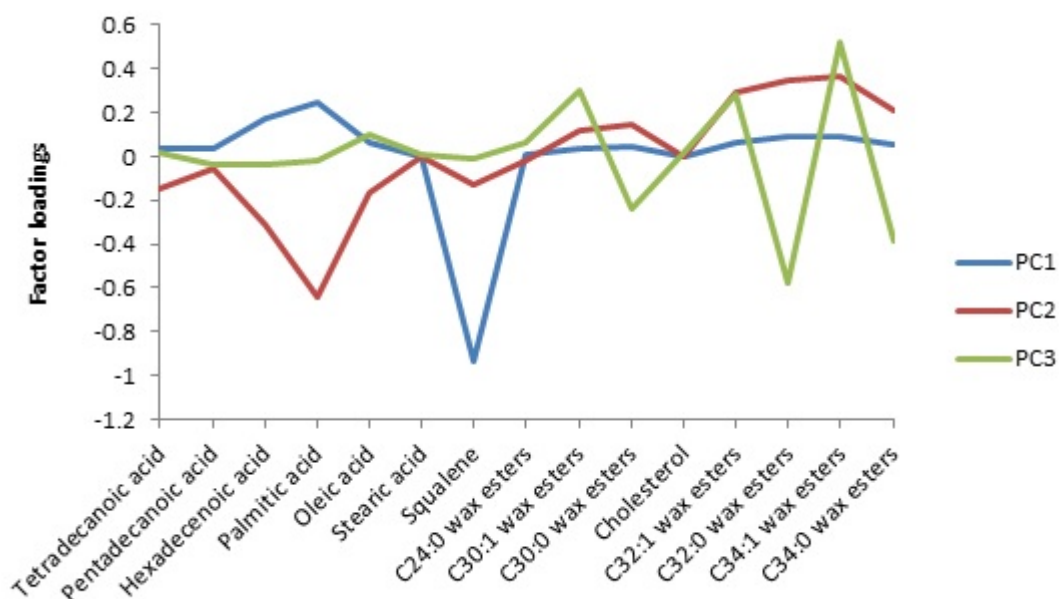


Figure 6: Factor loadings for the first 3 PCs

It has been suggested that the use of skin products may affect the consistency of latent fingerprint composition. In this investigation, no trends were observed that could be related to changes in use of skin products over 29 days, as three of the four donors reported consistent recent use. Interestingly, chromatograms of samples from an adult male donor collected 10 weeks apart as part of two other, separate investigations were found to differ noticeably in appearance (Figure 7). The donor had used skin products within 12 hours prior to the first sampling, but not the second. The samples collected after recent skin product use contained a number of exogenous compounds. Notably, stearic acid and several wax esters utilised in the PCA model, particularly myristyl myristate, were present in significantly higher proportions than encountered in most other samples. Conversely, the samples that contained no skin products contained a higher proportion of other free fatty acids. This observation indicates that irregular use or changes in habit (i.e. frequency or type(s) of products used) may significantly alter fingerprint composition to the point where samples taken from the same individual cannot be identified as such. This supports the conclusions drawn by

Gallagher *et al.* in this regard [63]. The potential of skin products to ‘mask’ the inherent composition of a fingerprint significantly hinders any prospect of using compositional profiling for identification purposes.

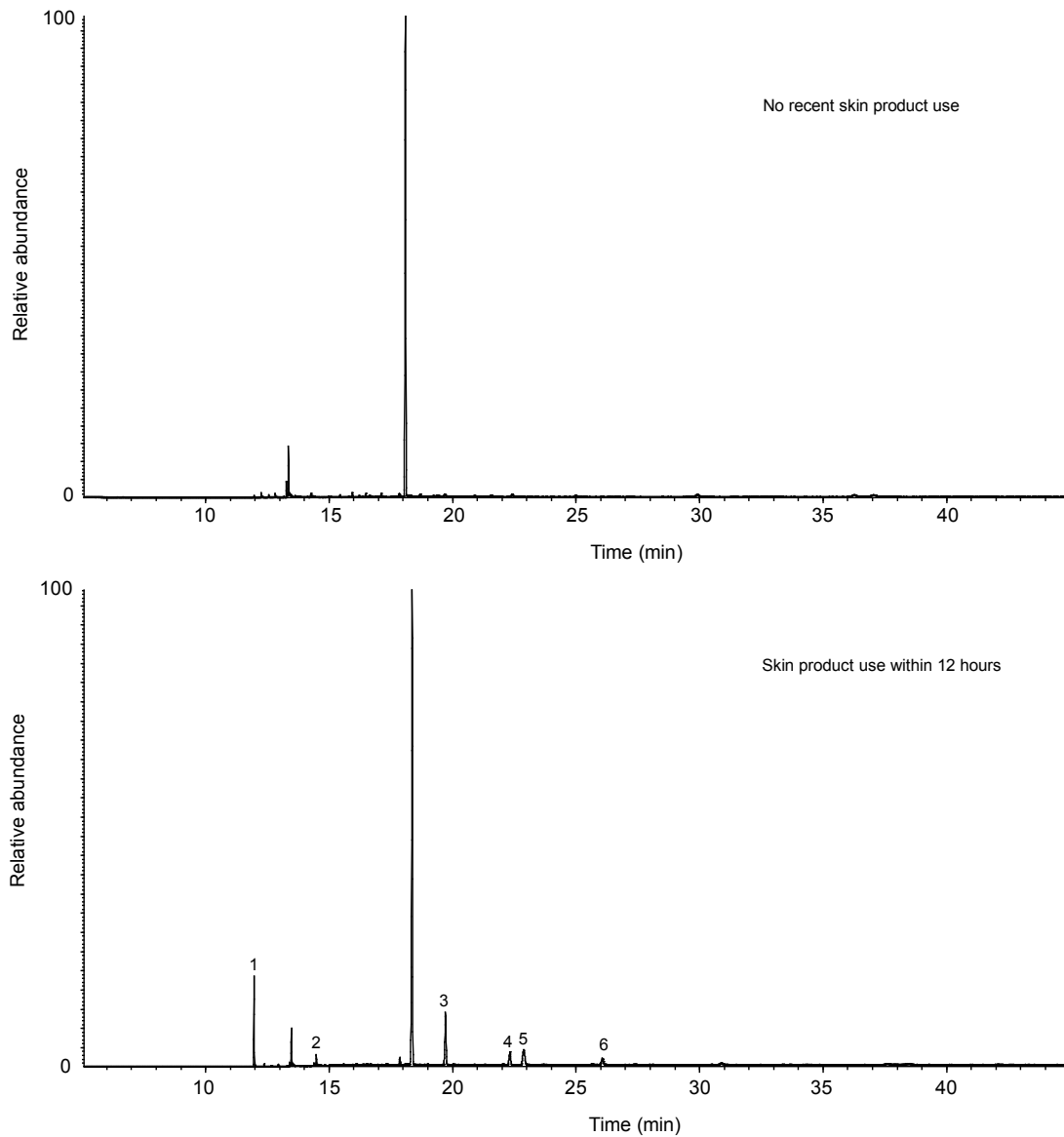


Figure 7: TICs of fingerprint samples collected from an adult male donor showing identified peaks related to use of skin products 1. Unknown compound, 2. Stearic acid, 3. Myristyl myristate, 4. Myristyl palmitate, 5. Vitamin E acetate, 6. Myristyl stearate

Inter-donor variation

The results of the above investigations indicate that differences in initial fingerprint lipid composition are greatly influenced by inter-donor variation. This investigation aimed to determine if significant differences could be observed in the composition of fingerprints collected from a large number of donors, and if these differences could be attributed to traits such as age or sex. Samples

were collected from 116 donors, ranging from 8 – 84 years of age, over a six month period. The time, date and location of sample collection varied at the convenience of the donors. A summary of the donor population demographics is outlined in Table 4. To avoid over or underrepresentation, donor numbers were kept as equal as was feasible for each age and sex category (6 – 7 individuals per group), with the exception of children, and donors over the age of 60. Due to difficulties in accessing donors of these ages, no quota was set on the number of donors from these age groups.

Table 4: Demographics of the inter-donor variation donor population

Donor age (years)	Male	Female	Total
0 - 9	10	8	18
10 -19	13	13	26
20 - 29	7	7	14
30 - 39	6	6	12
40 - 49	6	7	13
50 - 59	7	7	14
60 - 69	4	5	9
70 - 79	3	2	5
80 - 89	3	2	5
Total	59	57	116

Samples collected from some donors appeared to contain very little lipid material, such that only squalene and some fatty acids were visible in the chromatograms. This was seen consistently in additional samples collected from several of these donors over multiple consecutive days. Sample collection from these ‘weak’ donors was subsequently modified so that donors were asked to charge the middle three fingers of both hands, and deposit fingermarks from each hand onto the three filter papers provided. This modification was found to improve the detection of several major lipid components. This ‘double sampling’ procedure was also employed in situations where resampling was not possible (i.e. sample collection at public events). The analysis of samples from donors under 15 years old proved especially difficult, as samples collected from donors of this age group often contained very little analysable lipid material, producing blank chromatograms, or chromatograms that only contained squalene. As children’s fingermarks are known to contain far less material than adults’ [5, 6], the collection protocol was modified further to collect up to 6 charged fingermarks per

filter paper. Despite this measure, with the exception of two of the oldest children, many of the samples were found to contain only squalene and palmitic acid in detectable quantities (Figure 8), while no fingerprint material at all was detected in many others. As resampling from donors who only afforded blank chromatograms was often not practical, the data from 33 donors (including all donors under the age of 10) were not included in the PCA model.

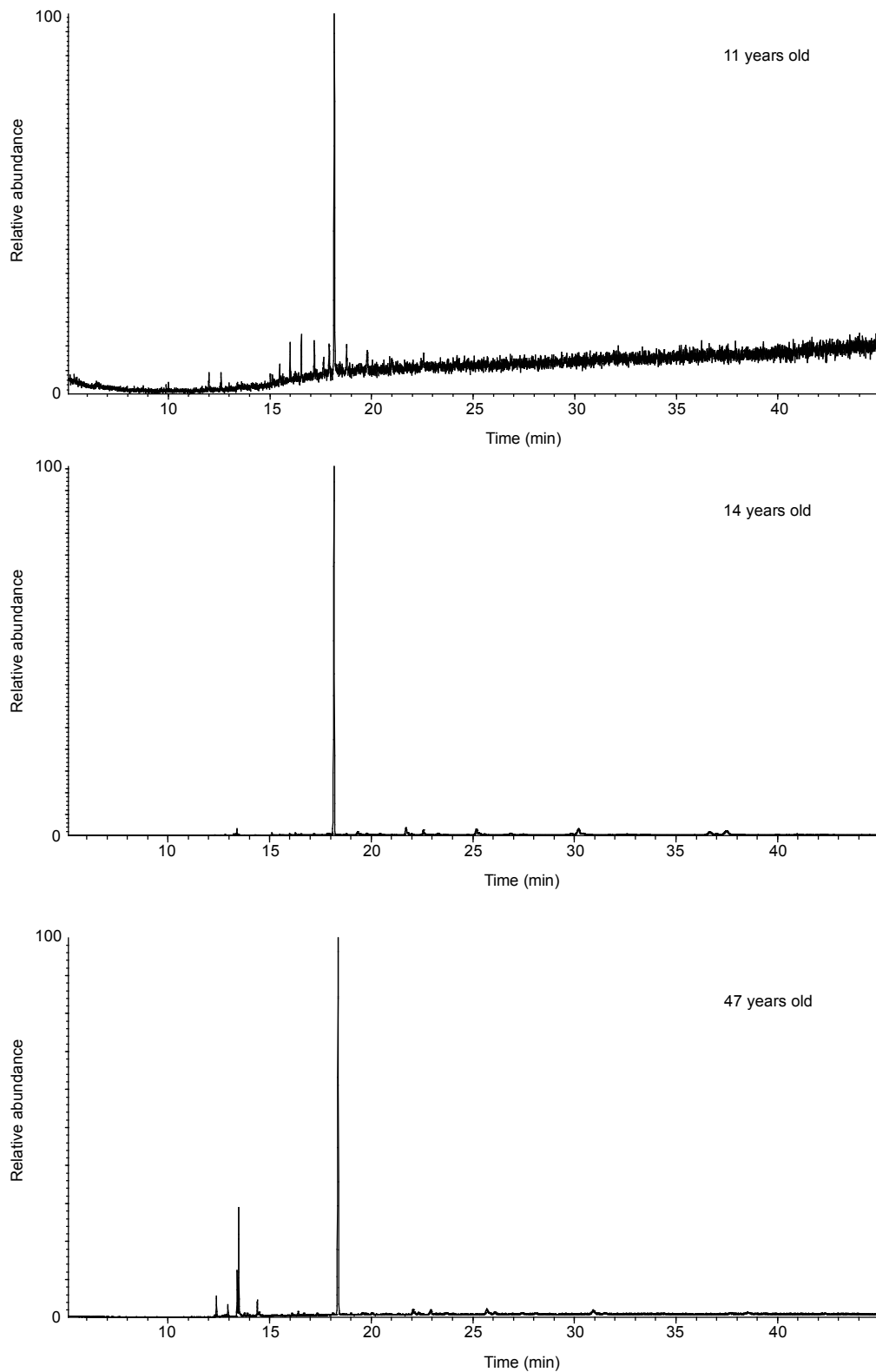


Figure 8: Comparison of TICs of samples collected from female donors of various ages

It should be noted that while sampling issues with weak donors could be partially overcome by deliberately 'overloading' the filter papers with several charged fingermarks, other studies into latent fingermark lipids have employed a method in which the sample extract is evaporated under

nitrogen to obtain a more concentrated sample [7, 10, 15, 24]. Such an approach may be beneficial in the analysis of fingermarks of young children and other weak donors; however, as described above, there are a number of considerations associated with such. Analysis of compounds from eccrine and epidermal sources might be more relevant to the composition of children's fingermarks, which typically do not contain significant amounts of sebaceous lipids [3, 64, 65].

PCA was performed on the dataset (216 chromatograms), revealing that 98.36 % of the total variance within the dataset was accounted for in the first 5 PCs (Figure 13). Scores plots were generated using a variety of combinations of the first 5 PCs, in order to determine the influence of PC4 and PC5 on the dataset, however no additional discrimination was gained. This is not unexpected, as the fourth and fifth PCs only account for 1.99 % and 1.04 % of the variance, respectively, and are unlikely to impact upon sample projection, given that the donor population in this investigation is much greater and more diverse than those described above.

Examination of the scores plot constructed using the first 3 PCs (Figure 9) showed that samples could not be visually discriminated by either individual donors or as a function of donor traits, as the samples were projected too close together. It is not altogether surprising that samples were not separated as a function of donor traits, as these factors are not mutually exclusive. The combined influences of donor traits is a major obstacle in attempting to correlate fingermark composition to donor characteristics [1]. Additionally, there are many other factors which may affect skin surface lipid composition which were not accounted for in this study. These are thought to include, but are not limited to, donor ethnicity, diet, metabolic disorders and use of some medications [1, 12, 24]. As such, it is difficult to determine compositional markers of traits, such as donor age, that are independent of other traits such as sex, metabolic disease, or the presence of exogenous contaminants. Based on the results of the intra-donor variation studies, and observed individual variation reported in the literature [1, 5, 6], it was thought that discrimination between individual donors might be possible. However, the use of a statistically significant donor population introduced a greater degree of overlap than had been observed in the previous models, such that adequate separation between donors was not achieved.

The factor loadings for the first 3 PCs were utilised to identify the compounds that contributed to the variance within the dataset (Figure 10). The loadings for PC1 was again almost identical to those discussed above, showing significant negative correlation to squalene, as well as some positive correlation to palmitic acid and hexadecenoic acid. Most variation of skin surface lipids appears to be related to the extent of triglyceride hydrolysis by skin flora, and the resultant fatty acid profiles; other sebum components such as cholesterol, sterol esters, and squalene have not been found to

exhibit significant variation [59]. The inter-donor differences in the relative amounts of palmitic acid, hexadecenoic acid and squalene, which comprise the most abundant endogenous components of most samples, may be attributed to this source of variation. The loadings for PC2 revealed significant positive correlation to stearic acid, as well as significant negative correlation to palmitic acid and some negative correlation to squalene. PC3 was found to have significant positive correlation to palmitoleic acid, and some negative correlation to palmitic and stearic acid. A high relative abundance of stearic acid in the fingermarks from one donor resulted in these samples being projected separately from the main cluster, along PC2. Examination of the chromatograms from this donor showed that these samples contained few endogenous components, and vitamin E acetate as a major component, indicating that stearic acid may be present as an ingredient of skin products.

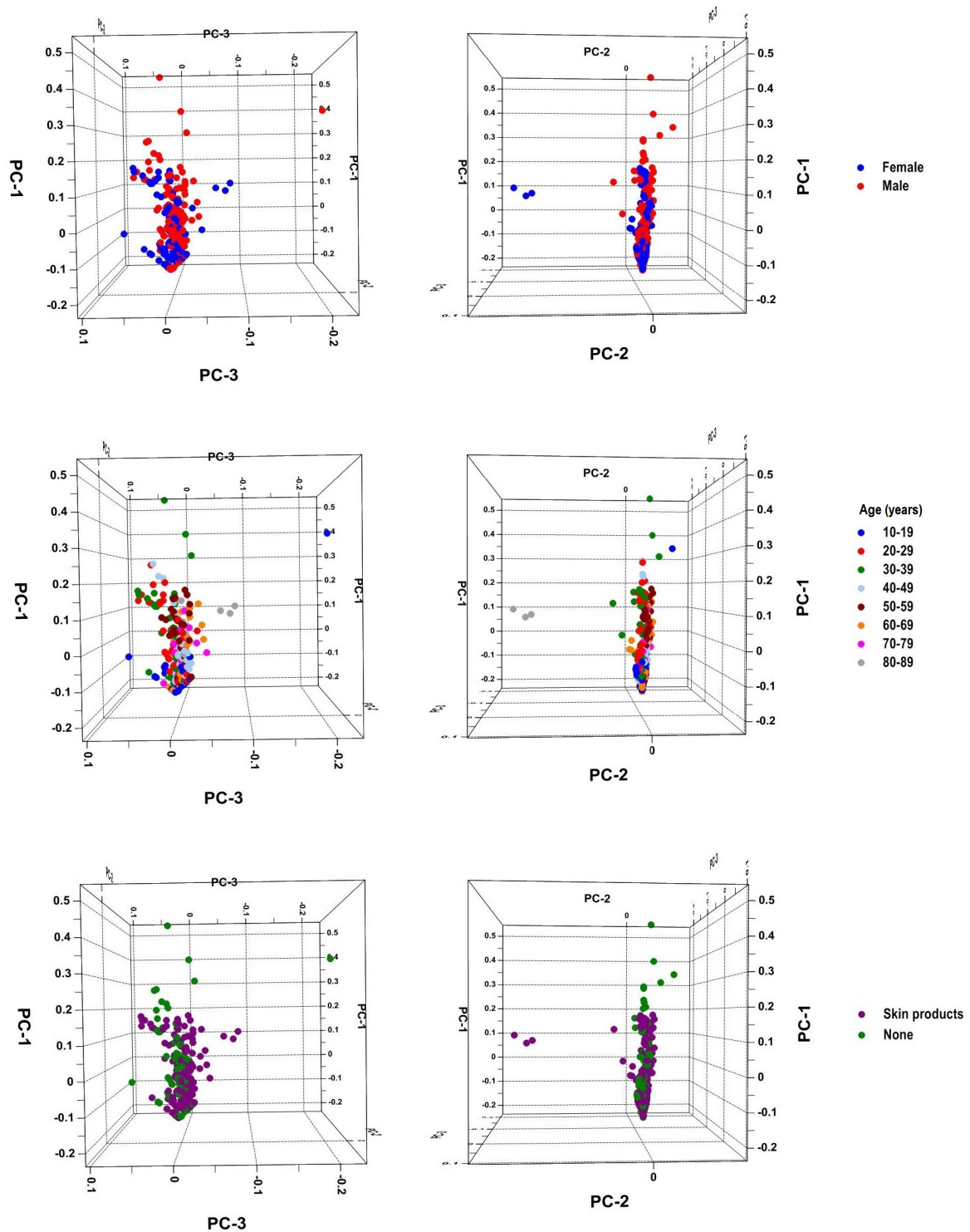


Figure 9: 3-dimensional scores plot generated from the first 3 PCs, demonstrating the distribution of fingermarks collected from 83 donors. Samples are coloured by biological sex (top), donor age in decades (middle), and recent use of skin products (bottom)

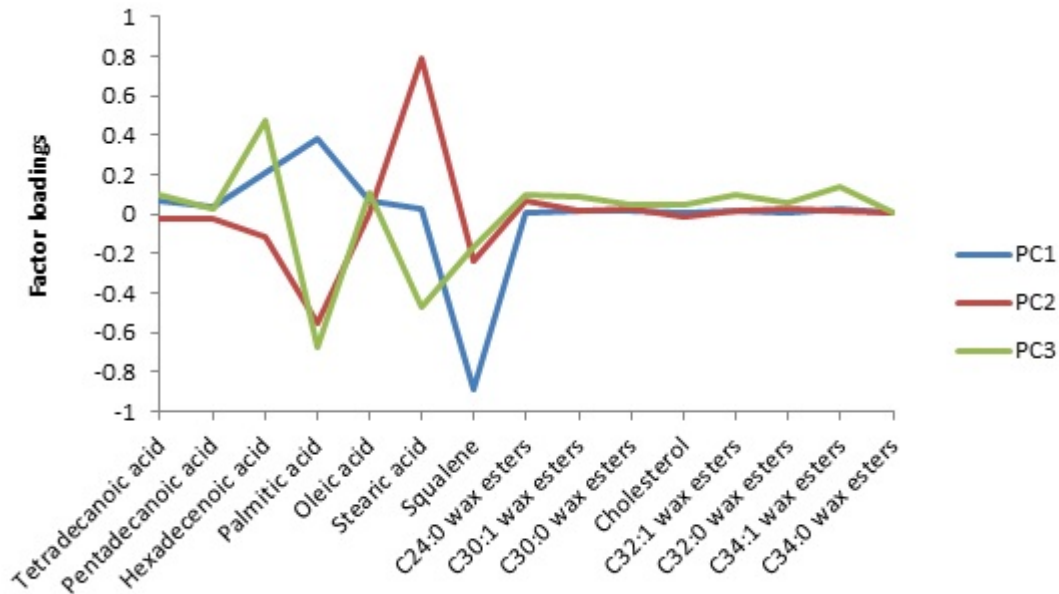


Figure 10: Factor loadings plots for the first 3 PCs

As seen in the intra-donor variation models, some donors exhibited good reproducibility, and replicate samples were projected close together, while those of other donors were projected significantly further apart. The reason for this is unclear at this point, as replicate samples were collected at the same time and in the same manner. However, several factors were noted during sample collection which may have affected how much fingerprint residue was deposited, including angle of contact (i.e. depositing material from the ends of the fingertip rather than the whole fingerprint), time spent charging fingerprints, size of donors' fingerprints and application pressure. These and other factors are thought to contribute to fingerprint composition, though the means of such are not completely understood. These factors may account in part for the observation that fingerprint composition varies with digit and handedness [25, 66].

The imperfect nature of latent fingerprints presents great complications to the proposition that chemical composition could be used for identification or dating purposes. Fingerprints from children and other poor lipid donors were difficult to detect using the presented method. Uncharged fingerprints, which may be more representative of those deposited by incidental contact at crime scenes, are likely to be similarly incompatible with the presented method.

It must be noted that the approach used in these investigations is exploratory in nature and there is potential for improvement. Penn *et al.* suggested that the profiling of entire chromatograms, rather than selected compounds, may achieve individual classification of volatile skin compounds [67]. While this may not be practical with latent fingerprints, considering the influence of exogenous

contaminants, utilising a greater number of endogenous compounds may reveal subtle differences that may enable better discrimination between donors or traits [7]. A more sensitive method of sample preparation, such as evaporating the extract and reconstituting with a smaller volume of solvent, may therefore be required for the detection of a larger number of compounds, despite the concomitant issues of potential contamination. As discussed above, the greatest source of compositional variation of skin surface lipids appears to be the hydrolysis of sebaceous triglycerides to their constituent fatty acids [59]. Measuring the relative amounts of triglycerides and free fatty acids may therefore enable greater discrimination. To do so would require modification of the methodology presented here, which is not amenable to the separation of triglycerides due to the maximum temperature limits of the columns available. Liquid chromatography may be a more suitable method for separation of triglycerides, though high temperature GC-MS separation and detection of all sebaceous lipid classes has been reported [32].

Conclusion

These results emphasise the complexity of latent fingerprint composition, and the challenges posed to current lines of research. While inter-donor variation in relative lipid abundances was observed, due primarily to squalene and free fatty acids, these differences were not sufficient to enable visual discrimination within a large donor population. There was also insufficient variation in the relative amounts of the selected lipid compounds to enable discrimination either between individual donors or their traits via multivariate statistics. Attempts to classify samples were further complicated by significant intra-donor variation. Additionally, inherent difficulties in obtaining reproducible fingerprint samples are recognised as possible functions of sample deposition, as well as compositional variation.

In this study, discrimination between donor traits based upon analytical chemical methods was not achieved due to the extremely complex nature of sebaceous lipids, which is in agreement with previous, smaller-scale studies by other researchers. It may be that interplay of donor traits and deposition factors as influences on fingerprint composition might never be completely understood due to their overlapping effects. While determining donor characteristics from latent fingerprint composition is an attractive concept, 'real world' factors such as the use of cosmetic products, recent washing of hands and other activities will mask any inherent differences in fingerprint composition. The analysis of fingerprint composition is likely to be of greater significance to the identification of common, abundant compounds as targets for detection methods, rather than classification for criminal investigations.

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