1   **Monitoring compositional changes during storage of the lipid fraction of fingermark residues**
2   **deposited on paper**

3   **Abstract**

4   Characterising the changes in fingermark composition as a function of time is of great value for
5   improving fingermark detection capabilities by understanding the processes and circumstances under
6   which target compounds become degraded. In this study, gas chromatography-mass spectrometry
7   was used to monitor relative changes in the lipids from latent fingermarks over 28 days. Principal
8   component analysis of the relative composition of 15 lipids in fingermarks showed that fingermark
9   age was a significant contributor to the variability observed in the data, but that inter-donor variability
10   was also significant. This was attributed principally to changes in the relative amounts of squalene,
11   which rapidly decreased in the fingermarks. It was also observed, however, that most fingermarks
12   exhibited relatively small changes in composition during the first seven days, followed by more rapid
13   changes up to 28 days. Significant inter-donor variation of both initial fingermark composition and the
14   rates and nature of loss processes was observed, which was reflected in the relative projection of
15   samples from different donors. Finally, samples stored with no exposure to light or airflow for 28 days
16   were projected significantly closer to the samples analysed on the day of deposition than those
17   exposed to light, due to the reduced photodegradation rate of squalene.

20   **1. Introduction**

21   In recent years, there have been several investigations into the changes in latent fingermark
22   composition that occur as a function of time. The stated aims have included the development of a
23   means to estimate the age of a fingermark for the purposes of criminal investigations [1-6], as well as
24   obtaining a better understanding of the processes of fingermark degradation that affect their
25   detection [1, 7], and the identification of compounds which remain stable over time (or are stable
26   degradation products) as potential targets for fingermark development [1, 8-10].

27   The lipid fraction comprises the more durable portion of latent fingermark residue (compared to the
28   water-soluble eccrine components), due to its hydrophobic and non-volatile nature. It is also highly
29   subject to compositional changes, and so it is this fraction of latent fingermarks which has been
30   studied most extensively in regards to changes in composition over time [4, 7, 9, 11-15]. Due to the
31   inherent variability of fingermark samples, a timeframe of the loss processes of the lipids has proved
32   difficult to characterise in detail; so far only broad trends have been identified [7, 9].

33   A recent study has explored the compositional variability of the lipid fraction of recently deposited
34   latent fingermarks [16]. It is recognised that this initial composition is not necessarily representative
35   of what is encountered in criminal investigations, as items may not be examined for latent fingermarks
36   until several days to weeks after deposition [17, 18]. Latent fingermark composition begins to alter
37   very soon after deposition, as evidenced by the quality of developed fingermarks of increasing age [1,
38   7, 11, 19, 20]. The lipid fraction of fingermark residue is considered to consist of two broad categories
39   of 'fragile' (fatty acids and triglycerides) and more stable 'robust' components (thought to include
40   large, insoluble proteins and lipoproteins) [21]. Physical developer, the most routinely used method
41   for detecting fingermarks on wetted paper substrates, is thought to target a mixture of compounds,
42   including the 'robust fraction', hence its ability to detect fingermarks that are several months old.
43   Conversely, detection treatments that target the 'fragile fraction', such as the lipophilic dyes Oil red O
44   and Nile red, perform comparatively poorly on fingermarks more than a few weeks old due to the

45 more short-lived nature of these compounds [22, 23]. Storage conditions, microbial activity, and the
46 application of development reagents are all thought to impact upon the rate and types of changes
47 that may occur [1-3, 7, 24, 25]. Environmental factors, including light exposure, substrate type,
48 temperature, humidity, airflow and immersion in water, are known to play a significant role in the
49 degradation rate; however, little is understood about their specific impact upon fingermark chemistry
50 [3, 4, 8, 12, 13, 19, 26-29]. The initial composition of a latent fingermark also has great influence on
51 its longevity [4, 7, 19].

52 Gas chromatography-mass spectrometry (GC-MS) has been utilised in several investigations into the
53 degradation of fingermark lipids as a function of time and environment [4, 7, 9, 25]. A crucial aspect
54 missing from these studies is frequent and consistent monitoring of compositional changes, to
55 establish whether or not degradation rates are uniform under constant environmental conditions, and
56 how degradation processes may vary between donors. Mong *et al*. analysed samples from all donors
57 at infrequent intervals of 0, 10, 30 and 60 days after deposition (or 0 and 30 days in the case of
58 children's samples) [9], while Archer *et al*. analysed samples at smaller time intervals of 1 – 12 days
59 over 33 days, but samples from only up to three of the five donors were analysed at any one time [7].
60 The required more comprehensive and frequent analyses will generate large multivariate datasets
61 that may give rise to issues in interpretation. Multivariate statistics can assist in extracting the
62 maximum amount of information from such datasets, by enabling the exploration of patterns and
63 trends in compositional changes over time in an objective and simplified manner [30].

64 This paper details an explorative investigation into the application of a previously described gas
65 chromatography-mass spectrometry method in conjunction with multivariate statistics to examine
66 the chemical changes undergone by latent fingermark lipids as a function of time [16]. Principal
67 component analysis (PCA) was performed on this data to assess the effects of inter-donor variation
68 and storage conditions on the types and rates of degradation processes, and therefore the projection
69 of samples within a three-dimensional scores plot. This approach enables the determination of which
70 compounds contribute the most variation over time, and hence are most likely to affect the detection
71 of older fingermarks.

72

73 **2. Materials and methods**

74 **2.1 Chemicals**

75 Tetradecanoic acid (Aldrich, USA), hexadecanoic acid (Fluka Analytical), hexadec-9-enoic acid (Sigma–
76 Aldrich, USA), octadecanoic acid (Aldrich, USA), squalene (Sigma–Aldrich, USA), cholesterol (BDH, UK),
77 tetradecyl hexadecanoate (Nu-Chek Prep, Inc, USA), tetradecyl hexadecanoate (Nu-Chek Prep, Inc,
78 USA), hexadecyl hexadecanoate (Nu-Chek Prep, Inc, USA), hexadecyl hexadecenoate (Nu-Chek Prep,
79 Inc, USA), octadecenyl tetradecanoate (Nu-Chek Prep, Inc, USA), USA), octadecyl tetradecanoate (Nu-
80 Chek Prep, Inc, USA), octadecyl hexadecenoate (Nu-Chek Prep, Inc, USA), hexadecyl octadecenoate
81 (Nu-Chek Prep, Inc, USA), octadecyl hexadecanoate (Nu-Chek Prep, Inc, USA) and dichloromethane
82 (Macron Chemicals, USA) were used as received. A set of standard solutions of the free fatty acids,
83 squalene, cholesterol and wax esters were prepared as individual solutions in dichloromethane at 50
84 ppm. All standard solutions were stored at −20 °C before and after analysis to prevent degradation
85 and solvent evaporation.

86 **2.2 Sample collection and storage**

87   Latent fingermark samples were collected from 8 donors on filter paper circles (25 mm qualitative
88   filter paper, Grade 1; Whatman, UK). The donors were selected based on the chromatograms of
89   fingermark samples provided in previous investigations [16], and also for the relatively small
90   differences in age. A variety of strong and weak lipid donors were chosen, as well as some known to
91   use cosmetic products regularly. A summary of the characteristics of the donor population is
92   presented in Table 1.

93   **Table 1:** Summary of donor information

Sex	Male (*n*)	Female (*n*)
	4	4
Age (years)	20 – 29 (*n*)	30 – 39 (*n*)
	6	2
Recent skin product use (<12 hours)	Yes (*n*)	No (*n*)
	7	1
Total	8	

94

95   Donors were instructed to briefly rub the tips of their middle three fingers of each hand on their
96   forehead or nose, and then press the fingertips of one hand gently to a filter paper circle for
97   approximately ten seconds, followed by the immediate deposition of fingermarks from the other
98   hand. Fourteen sets of sample replicates were collected in triplicate from each donor, during 3
99   sampling times over a period of 5 h, to provide a total of 336 fingermarks. A maximum of 5 sets of
100 samples were collected at each sampling time, with a period of at least 1 h in between sampling times,
101 to allow sebum to re-accumulate on the skin surface. Information regarding donor activity and
102 handled substances was collected at each sampling time.

103 One set of samples from each donor was analysed by GC–MS on the day of collection (within 2 h of
104 deposition) to obtain profiles of initial fingermark composition. Twelve sets from each donor were
105 placed in uncapped 20 mL glass vials (Gerresheimer, Germany), with all replicate samples in each set
106 stored in the same vial. Sample vials were placed in a tray and stored on a shelf in an office
107 environment at room temperature (21–23 °C) with exposure to light and airflow for up to 28 days. An
108 open vial containing clean filter papers was stored with the samples to provide analytical blanks for
109 each analysis time. The final sample set from each donor were stored in glass vials that were
110 completely wrapped and sealed in aluminium foil, and stored in a cardboard box adjacent to the open-
111 topped vials. These samples were stored alongside the samples in open vials for 28 days.

112 **2.3 Sample preparation**

113 From the samples stored in open glass vials, one randomly selected sample set from each donor was
114 analysed 2, 5, 7, 9, 12, 14, 16, 19, 21, 23, 26 and 28 days after deposition. The samples stored in foil-
115 wrapped vials were analysed 28 days after sample collection. Extraction of fingermark residue from
116 the filter papers was performed as described by Frick et al. [16]. Analytical blanks consisting of the
117 stored, clean filter papers were prepared and analysed with each set of samples. Chromatograms from
118 329 samples were used in the PCA dataset due to there being no detectable amounts of target lipids
119 in several samples from donor CB050 (all samples analysed 16 and 28 days after deposition, and one
120 sample stored in a foil-wrapped vial).

121 **2.4 Gas chromatography-mass spectrometry**

122 Chromatographic analysis was performed using a Hewlett–Packard 6890N series GC interfaced with
123 an Agilent 5975 inert mass selective detector as described by Frick et al. [16].

124 **2.5 Data analysis**

125 The data was pre-processed using Chemstation Data Analysis (Agilent Technologies, USA) by
126 background subtraction of all chromatograms, followed by manual integration of 15 selected peaks in
127 the total ion chromatograms (tetradecanoic acid, pentadecanoic acid, hexadecenoic acid,
128 hexadecanoic acid, octadecanoic acid, squalene, C28:0 wax esters, C30:1 wax esters, C30:0 wax esters,
129 cholesterol, C32:1 wax esters, C32:0 wax esters, C34:1 wax esters and C34:0 wax esters), which were
130 identified as described by Frick *et al*. [16]. Replicates from each donor were treated as individual
131 samples in the data matrix. Peak areas were normalised to the sum and autoscaled using Microsoft
132 Excel. Principal component analysis (PCA) of the data was performed using the Unscrambler® X 10.4
133 software (CAMO Software AS, Oslo, Norway).

134 **3. Theory**

135 Difficulties in interpreting the volume of data generated by this study (329 chromatograms) can be
136 overcome through the application of multivariate statistical methods, or chemometrics [31-33]. One
137 of the most widely used such methods is principal component analysis (PCA) [34]. PCA simplifies large,
138 multivariate datasets, such as chromatographic data, in an objective and reproducible manner [34-
139 37]. This is achieved by reducing data dimensionality through the transformation of multiple variables
140 from the original datasets into a reduced number of new, orthogonal variables known as principal
141 components (PCs) [34, 36, 38-40]. The first PC explains the largest percentage of variance within the
142 original dataset, and each subsequent PC describes a decreasing value of the remainder [35, 37, 38,
143 41]. Generally, only the first few PCs need to be examined to account for the vast majority of the
144 variance within the original dataset. These PCs may then be used to construct a scores plot: a 2- or 3-
145 dimensional visualisation of patterns and relationships within the dataset that may not be discernible
146 by examination of the original variables [33, 37-39, 42]. Further interpretation of the data is achieved
147 through comparison of the scores plot with the loadings plot, which indicates the variable(s) in the
148 original dataset which have the greatest influence on each PC [39, 43-45]. PCA is often used in
149 exploratory data analysis and in the construction of predictive models in conjunction with linear
150 discriminant analysis (LDA) [40-42].

151 **4. Calculation**

152 **4.1 Constant-sum normalisation**

153 Chromatograms were normalised to the sum of all integrated peak areas ($\sum A_i$) to overcome variation
154 in the amounts of residue deposited by donors due to differences in fingermark size or deposition
155 technique, as well as instrument response:

156 $$Normalised\ peak\ area = \frac{A}{\sum_{i=1}^{n} A_i}$$

157 Donors often do not reproducibly deposit an impression of the entire fingermark pattern, which may
158 contribute to reproducibility issues. Normalisation to the sum was compared to normalisation to the
159 square root of the sum of squares, which can further reduce the influence of this variability on the
160 PCA models. The scores plots showed no significant differences in projection. As such, the former
161 method was used.

162 **4.2 Autoscaling**

163 Autoscaling was performed on all normalised peak areas in order to reduce the influence of the most
164 abundant and variable compounds (i.e. squalene, hexadecanoic acid and hexadecenoic acid) on the
165 dataset:

166
$$Autoscaled\ peak\ area = \frac{(x - \bar{x})}{S_x}$$

167 Here, x represents the normalised area of each peak in a sample, and $\bar{x}$ and S_x the corresponding
168 mean and standard deviation across the dataset, respectively.

169 **4.3 Euclidean distance**

170 Distance plots were constructed from the datasets of each donor using the scores from the first two
171 PCs as x, y coordinates for each sample. The centroid (i.e. the mean coordinates) of the day 0 replicates
172 was used as the point of origin for each distance plot $(\bar{x}, \bar{y})$, and the distances between each of the
173 samples and the centroid were calculated using the formula:

174
$$Distance = \sqrt{(x - \bar{x})^2 + (y - \bar{y})^2}$$

175 Distance plots were constructed using the average distance for samples from each day of analysis,
176 with error bars showing the range.

177 **5. Results and discussion**

178 Major compositional changes with increasing sample age were found to be common to all donors with
179 regards to the relative abundance of the 15 compounds of interest during the 28 day period. The most
180 obvious change was the marked reduction in peak height of squalene, such that hexadecenoic acid
181 and hexadecanoic acid became the predominant compounds (Figure 1). The precise timing and the
182 extent of this change appeared to be dependent on the initial composition of the fingermark; samples
183 collected from donors with a naturally low fatty acid to squalene ratio exhibited this change sooner
184 than those from donors with a much higher ratio.

185 The rapid reduction of squalene is consistent with observations made by Archer *et al*. [7], with the
186 exception that squalene was still detected in samples from 7 of the 8 donors after 28 days, whereas
187 Archer *et al*. reported that squalene could not be detected in any samples stored under constant light
188 after 20 days. The experimental conditions used by Archer *et al*. utilised constant, direct illumination
189 to contrast with the effects of storage in complete darkness, whereas in this study, samples were
190 stored under fluorescent office lights set to switch off when the office was unoccupied, thereby
191 providing a more typical diurnal exposure. Samples were stored in an office environment rather than
192 a laboratory to mimic the conditions on which fingermarks on documents or other paper substrates
193 might commonly be stored, and to prevent contamination from reagents [9]. This difference in
194 illumination conditions may account for the differences in squalene degradation, given that squalene
195 undergoes photo-oxidative degradation [46].
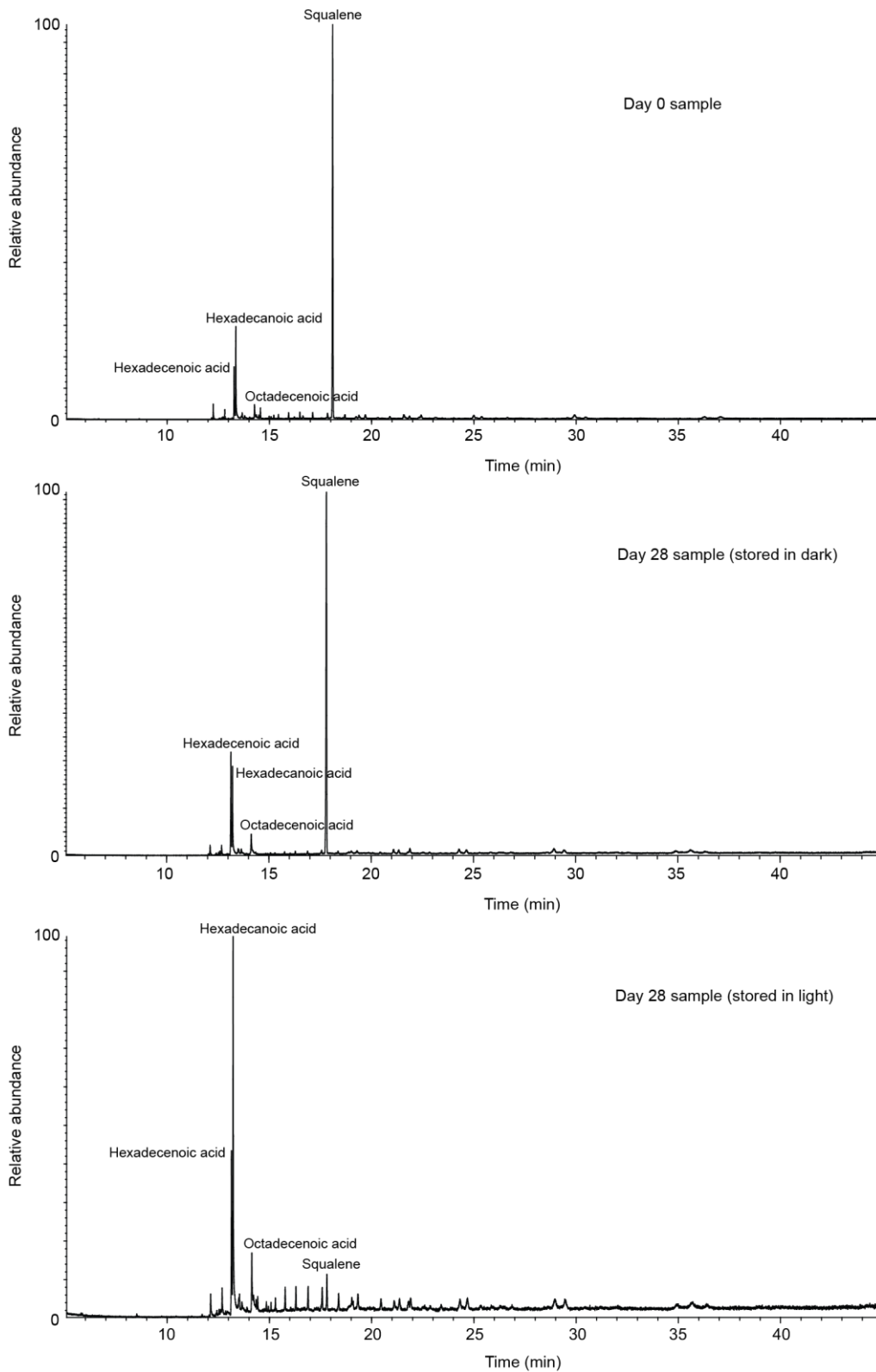
Figure 1: Total ion chromatograms representing changes in relative abundances of compounds detected in fingermarks from a single donor

The relative peak areas of the free fatty acids themselves were highly variable, with a general trend towards an overall increase at the end of the 28 days. The proportion of the peak areas of the

201   saturated wax esters to their monounsaturated counterparts appeared to increase. It is unclear from
202   only a visual inspection of the chromatograms as to whether these represent actual compositional
203   changes related to the age of the fingermark [9]. As repeated analyses of a sample over the 28 days
204   was not possible, due to the destructive nature of the extraction and GC-MS method, it must be
205   assumed that all samples from each donor have an identical initial composition as the sample
206   collection strategy was designed to minimise intra-donor variation. However it must be borne in mind
207   that variation may still be present. Numerous studies have shown that latent fingermarks can exhibit
208   significant intra-donor variation, which impacts upon reproducibility. GC-MS studies into the ageing
209   of latent fingermarks, particularly for age estimation, that utilise absolute quantification methods are
210   frequently complicated by difficulties in obtaining reproducible, homogenous samples for
211   comparative purposes [7, 9, 25, 47].

212   PCA of the total dataset (329 chromatograms) revealed that 83.04 % of the variance within the dataset
213   was accounted for by the first 5 PCs (Figure 2). The scores plot constructed from the first 3 PCs (Figure
214   3) revealed that significant changes in composition occurred over the 28 days of the investigation. PCA
215   of the normalised and autoscaled data was compared against PCA following normalisation to the sum
216   only, which revealed that 99.23% of the variance within the dataset was accounted for by the first 5
217   PCs. While the cumulative variance of the autoscaled data was lower than that of the data processed
218   using only normalisation, it was found that autoscaling prior to PCA enabled subtle trends in the
219   dataset to become more apparent, as the influence of squalene, hexadecanoic acid and hexadecenoic
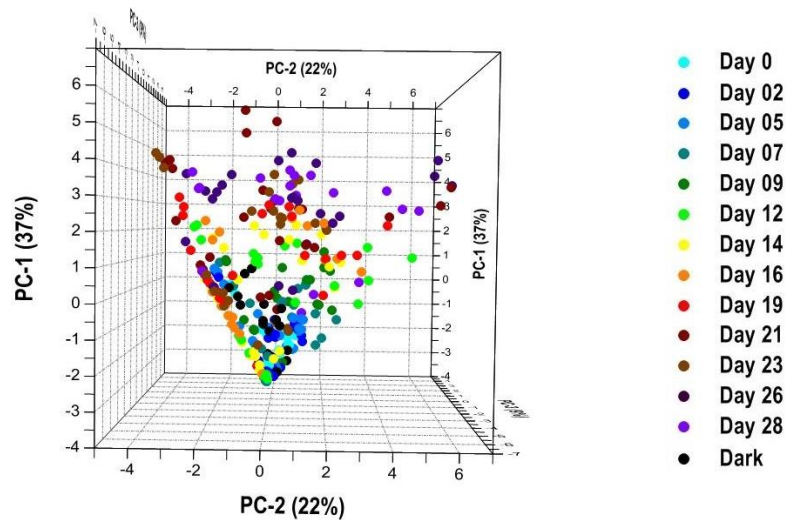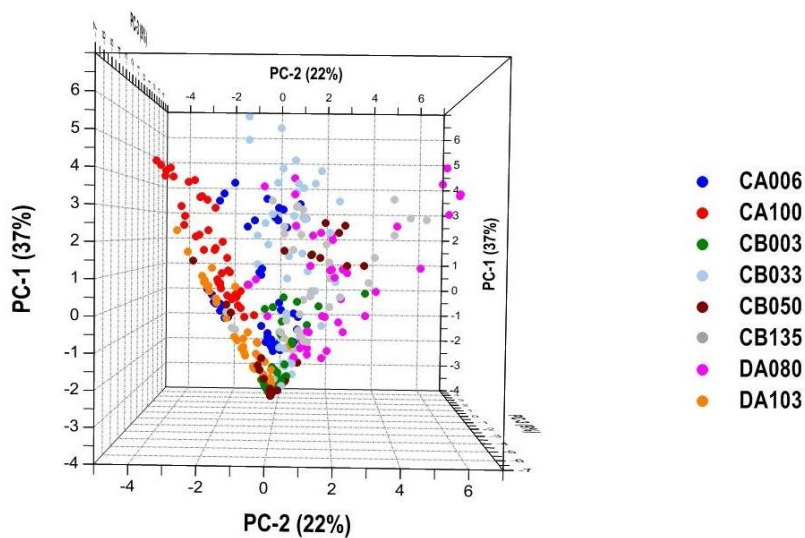220   acid were reduced.
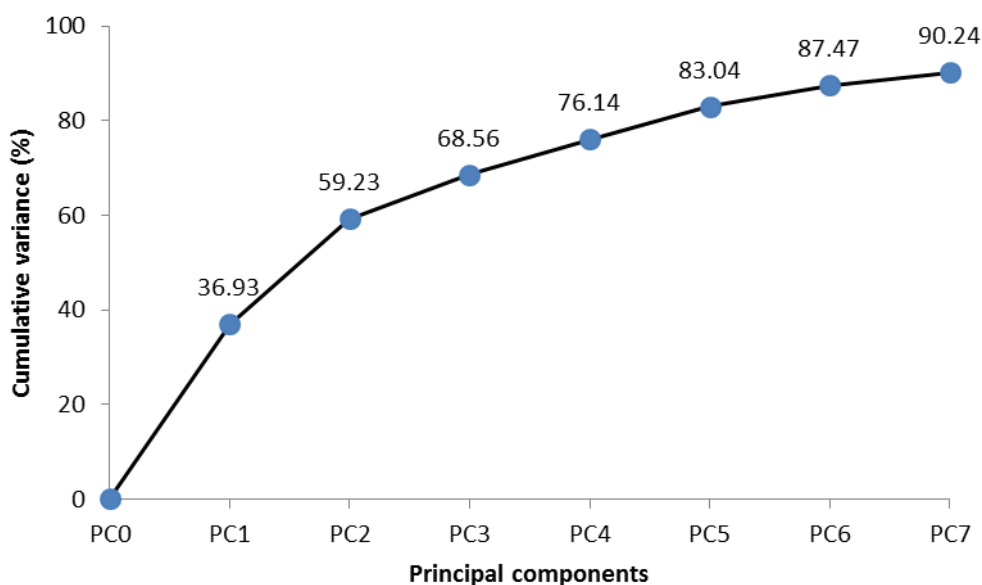
221

222   **Figure 2**: Scree plot depicting the variance the dataset accounted for by each PC

223

224

225

226

Figure 3: 3-dimensional scores plot generated from the first 3 PCs, demonstrating the distribution of fingermarks of increasing age collected from 8 donors. Samples are colourised by sample age in days (top) and individual donors (bottom)

Samples from all 8 donors were projected primarily along PC1, with scores increasing with sample age. The 'starting point' of this scatter (i.e. lowest scoring samples on PC1) was different for each donor, which is likely due to inter-donor compositional differences. Over the 28 day period, samples from each of the 8 donors also became scattered in different directions along the second and third PCs. With increased sample age, replicate samples were projected further away from each other, indicating that disparities between replicates became exacerbated by degradation processes.

The factor loadings for the first 3 PCs (Figure 4) were utilised to identify the compounds that contributed most to the variance within the dataset. The loadings plot for PC1 revealed significant negative correlation to squalene, as well as positive correlation to the free fatty acids and the wax esters of ≥30 carbon units. Hence, more recently deposited samples, which contained relatively large amounts of squalene and relatively small amounts of the fatty acids and wax esters, were assigned

negative scores on PC1, while older samples had increasingly positive scores as squalene concentration decreased.
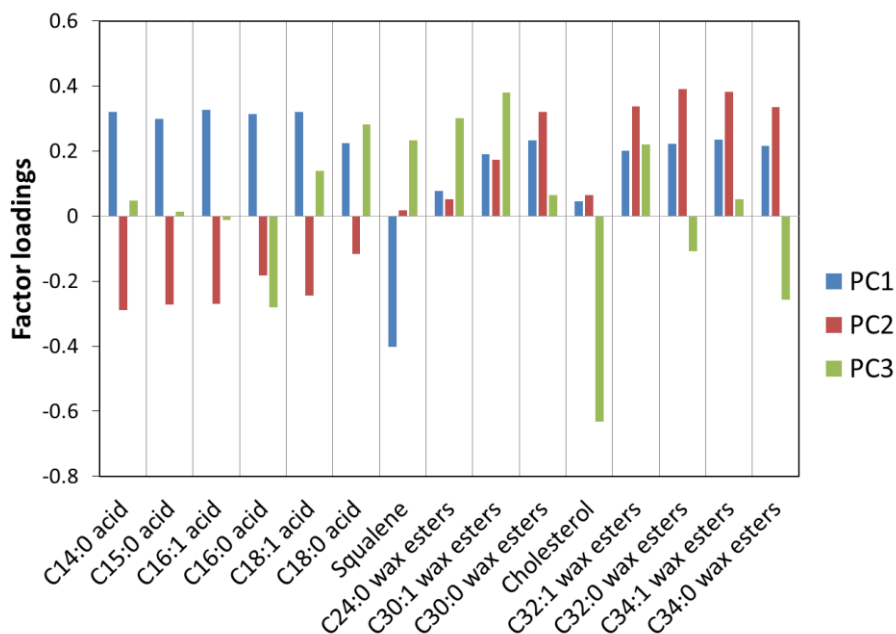
Figure 4: Factor loadings plots for the first 3 PCs

The relative amounts of squalene, fatty acids and wax esters are reflective of compositional changes in samples of increasing age, as well as inter-donor variation, as evidenced by the projection of the day 0 samples. Consequently, the total dataset comprising all 8 donors is not well suited to estimating fingermark age. The loadings plot for PC2 revealed significant negative correlation to the free fatty acids, and significant positive correlation to several of the wax esters. Examination of the scores plot, in comparison with the appearance of the chromatograms, indicates that sample projection along PC2 is influenced by a combination of sample age and inter-donor compositional differences related to the relative proportions of the more volatile components. For example, day 0 samples from donors CA100 and DA080 contained relatively high levels of free fatty acids and wax esters, respectively, compared to other donors. The loss of squalene accounts for the increasingly large difference in scores assigned to samples from the two donors over time, as this would increase the relative amounts of the other components. The loadings plot for PC3 revealed significant positive correlation to octadecanoic acid, squalene, and the C24:0 and C30:1 wax esters, some negative correlation to hexadecanoic acid and C34:0 wax esters, and a significant negative correlation to cholesterol. Due to the differences in projection of older samples between donors, it is unclear from examination of the scores plot as to whether sample distribution along the third PC is due to sample age, inter-donor variation or a combination of the two. Samples from most donors obtained scores of approximately 0 along this PC, while older samples from two female donors, CB050 and CB135 attained increasingly negative scores, indicating a larger proportion of cholesterol. The factor loadings of this PC may be influenced by exogenous contaminants in the form of cosmetic or other skin products.

The changes in sebaceous lipid composition over time has a marked impact on the ability to assign latent fingermarks to a particular individual or specific characteristics such as age or biological sex. With increasing age, samples from the total dataset are increasingly scattered, rather than forming separate groups. While the points for some donor's fingermarks may follow a clear linear trend primarily along PC1, such as donor CA100, those from others, such as donor CB135, are more scattered. The inconsistency of fingermark degradation poses enormous difficulties in establishing a

271 method of estimating fingermark age, but does demonstrate that lipid degradation can be expected
272 to significantly affect any kind of classification model.

5.2 Effect of storage conditions on degradation rate

274 Storage conditions had a significant impact upon squalene loss mechanisms, as samples stored in foil-
275 wrapped vials for 28 days did not exhibit the dramatic decrease of this compound seen in the samples
276 stored in open vials for the same length of time (Figure 1). Samples that had been stored in foil-
277 wrapped vials for the duration of the investigation were generally projected very close to the day 0
278 samples for each donor in the PCA scores plot (Figure 3). This observation is consistent with previous
279 reports that the decrease in squalene concentration in latent fingermarks is accelerated by exposure
280 to light, compared to those stored in dark conditions [1, 7]. That these samples were not projected
281 separately from the day 0 samples along the second or third PCs further indicates that these PCs reflect
282 inter-donor variation rather than degradation processes involving free fatty acids, cholesterol or wax
283 esters. Due to time and practicality constraints, additional samples were not collected to further
284 investigate the effects of sample storage conditions on sample projection throughout the 28 day
285 period.

286 Difficulties in the detection of older fingermarks have often been linked to environmental conditions,
287 as well as fingermark age. Whether or not a latent fingermark was recently deposited has been
288 inferred from the quality of ridge development with powdering methods [6, 12], but this is a highly
289 unreliable method as high quality fingermarks up to 6 months old may be detected in such a manner
290 [12, 48]. It is shown here that the environment that a fingermark has been exposed to, including
291 factors such as exposure to light, has a significant effect on the rate of degradation of certain
292 compounds, which may account for some of these inconsistencies. The effects of other factors such
293 as temperature and humidity have been speculated upon, but not as thoroughly investigated [7]. The
294 effect of temperature is an important factor that needs to be explored further, particularly considering
295 the substantial effect that higher temperatures have on the detection of children's fingermarks [29,
296 30]. Additionally, substrate type has been shown to have a marked effect on fingermark longevity,
297 with faster degradation on nonporous substrates than porous ones [4].

5.3 Inter-donor variation

299 As discussed above, the disparate projection of samples from the 8 donors caused by inter- and intra-
300 donor variation, as well as sample age, creates difficulties in interpreting the dataset in its entirety.
301 The extent to which samples from each donor are projected along PC1 in the scores plot generated
302 from the total dataset indicates that the rate of change in fingermark composition over the 28 day
303 period is also subject to inter-donor variation. Subsequently, samples from each donor were treated
304 as individual datasets to better enable examination of compositional changes as a function of time
305 and storage conditions, independent of inter-donor variables.

306 When PCA was performed on samples from a single donor, the first two PCs accounted for 52–95% of
307 the variance within each dataset. Data for one donor (CA006) is provided here as an example of the
308 results discussed in this section. The scores plots constructed from the first 2 PCs (Figure 5), were
309 broadly similar to the scores plot generated from the total dataset. Samples were projected primarily
310 along the first PC, with older samples again attaining increasing scores. When fingermark degradation
311 was examined on a per donor basis, other compositional changes became more evident. Examination
312 of the scores plots revealed that samples from several donors appeared to exhibit a 'stable period'.
313 This period lasted approximately 7 days for most donors, with only small changes in the
314 chromatograms. However, this stable period was as short as 2 days and as long as 14 days for some

donors. This indicates that the extent and rate of lipid loss varies between individuals, which is in part a reflection of the initial composition. Other groups in the scores plots were formed by samples ranging from 21–28 days old from some donors, and additional clusters of samples of intermediate age were also observed. Samples that had been stored in foil-wrapped vials were generally projected close to samples analysed within up to a few days following deposition.
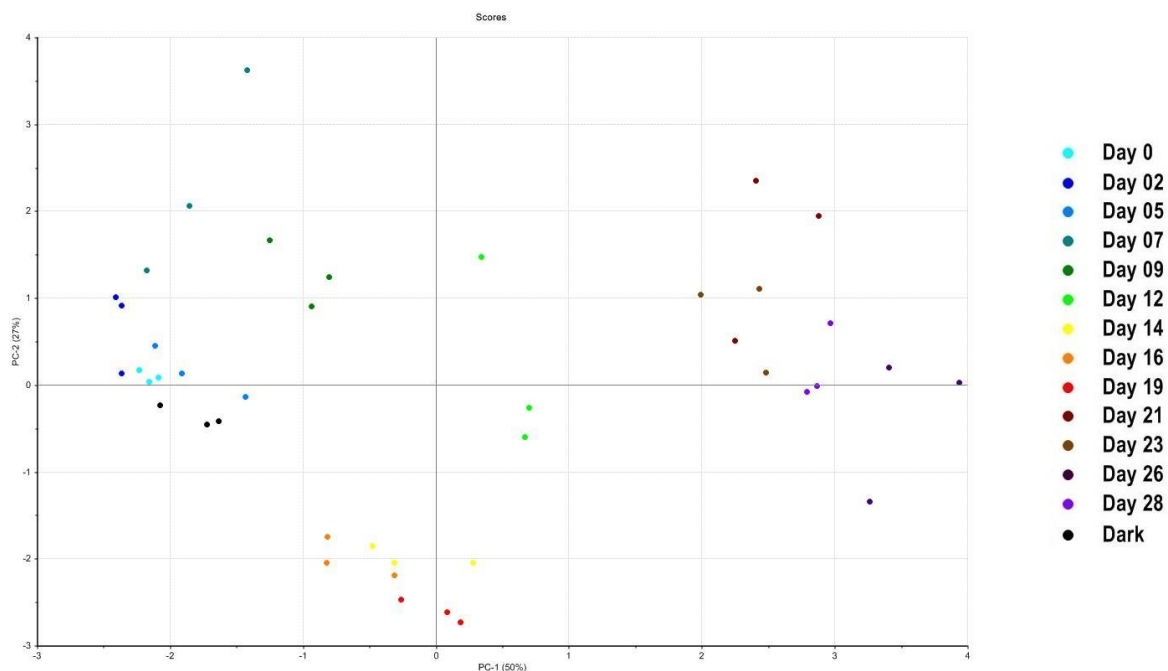

Figure 5: 2-dimensional scores plot generated from the first 2 PCs, demonstrating the distribution of fingermarks of increasing age of samples from donor CA006

The factor loadings for the first 2 PCs from the PCA analysis of samples from single donors were utilised to identify the key compounds contributing to the variance within each dataset (Figure 6). For all but one donor, the loadings plots for PC1 revealed significant negative correlation to squalene and positive correlation to several of the fatty acids and wax esters, emphasising the influence of squalene loss as a predominant degradation process over the 28 days of this study. The loadings plots for the second PC revealed different chemical changes between donors, mainly fluctuations in the relative amounts of free fatty acids and wax esters, with some influence from squalene and cholesterol in certain donors. In some donors, there appeared to be a relative increase in wax esters, in conjunction with increases and/or decreases in several fatty acids, particularly hexadecanoic and octadecanoic acids, over the 28 days. For four donors, there was no significant variation or clear trend in sample distribution along this PC. Archer et al. observed that the amounts of long chain fatty acids first decreased, and then increased in ageing fingermarks, and concluded that this may be indicative of two competing mechanisms of degradation, one acting on the fatty acids themselves, and another acting on wax esters or triglycerides [7]. It should be noted that wax esters and triglycerides were not detected using the methodology employed by Archer *et al*., making it difficult to be certain about the source of the fatty acids [7]. Conversely, Weyermann *et al*. reported no significant changes in fatty acid concentrations over 30 days [4]; however, these samples were stored in complete darkness.
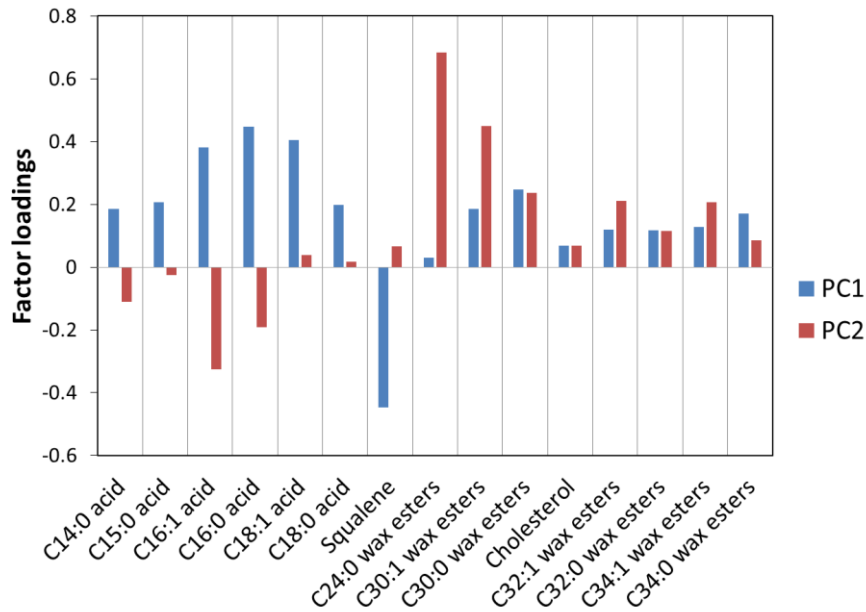
**Figure 6:** Factor loadings for the first 2 PCs of samples from donor CA006

The rate and nature of fingermark degradation, as well as differences in rate between donors, were further investigated using Euclidean distance plots. Fingermarks from all donors that were stored in the light followed a general trend of increased distance from the centroid (the mean coordinates of the day 0 replicates) with time, which for 5 of the donors appeared to be linear (Figure 7). The samples from the other three donors produced more exponential distance plots. It is unclear, based on these data, as to why the nature of the rate of compositional change varied as such amongst the 8 donors. The samples stored in the dark for 28 days were plotted a significantly closer distance to the centroid than those stored in the light for the same period of time. In some donors this distance was virtually indistinguishable from the day 0 samples, while in others there was a greater difference, but still much closer to the centroid than the samples stored in light.
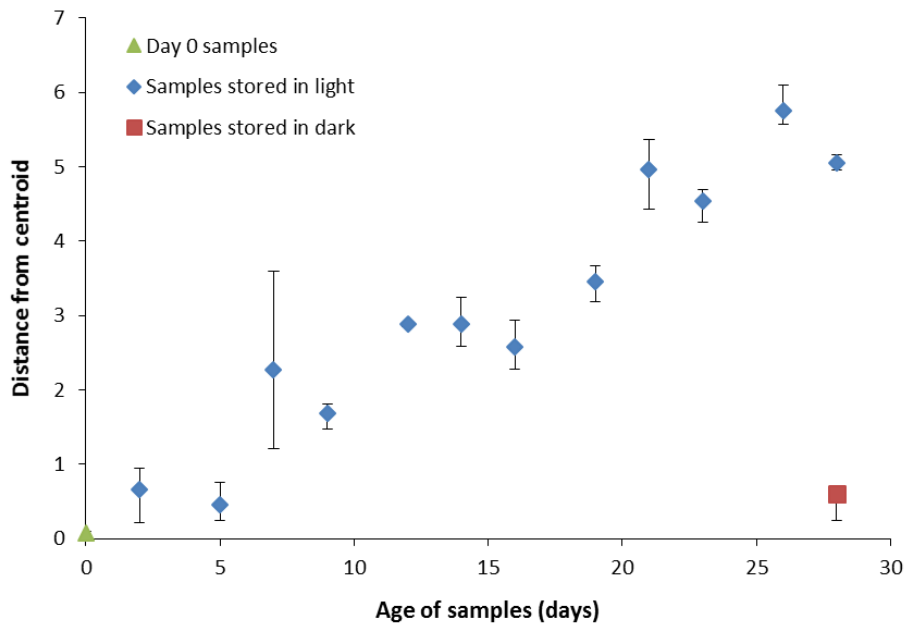
Figure 7: Distance plot constructed from scores of first two PCs, depicting rate of total compositional change over time of samples from donor CA006

The impact of intra-and inter-donor variation is such that a ubiquitous timeline for latent fingermark degradation processes is difficult to establish. For example, compositional differences between donors may also have some impact on whether squalene can still be detected in a fingermark after a significant period of time [7, 9]. Girod *et al*. proposed that due to the significant variability in fingermark composition, and its resultant effect on degradation rates and processes, that individual-specific regression curves should be constructed as required to estimate fingermark age [25]. However, this approach is impractical in an operational context. Firstly, an identifiable fingermark needs to be obtained, so that the corresponding individual may be located and be present to provide fingermark samples in order to construct a degradation model [25]. Secondly, as demonstrated here and in numerous other studies, storage conditions can have a marked effect on degradation rate; therefore, a lack of knowledge regarding the environment in which a fingermark has been stored will complicate the comparison of the questioned fingermark to a degradation curve. Given the impact that intra-donor variation may have on degradation, even if the above factors can be accounted for, age estimation of latent fingermarks may still be prone to large uncertainties [7].

The main implication of these results is that the rates and types of changes in fingermark composition with age are influenced by numerous variables. Further studies are required to account for additional environmental factors to explore the impact of storage conditions on compositional variation. Exposure to varying degrees of light, temperature, humidity, immersion in water, airflow, microbial action, and presence of contaminants are only some of the factors which can affect lipid degradation. Such studies would assist in providing detailed information regarding fingermark degradation processes and possible new target compounds in degradation products.

6. Conclusions

The number of lipid species present in latent fingermarks presents a great challenge not only to research into compositional variation between individuals, but also regarding their degradation as a function of time and environment. Difficulties in obtaining reproducible samples from individual donors further complicated the distribution of older samples within scores plots. Although work to fully explore the potential of utilising fingermark composition for development of classification systems for donor traits, individualisation or fingermark age could be envisaged, such efforts would be complicated by the uncontrollable variables influencing fingermark composition. A preliminary comparison into the effects of storage conditions emphasises that storage conditions can have significant effects on the rate of degradation. While only a limited number of environmental conditions were explored, this reinforces that there are many challenges facing the development of detection methods that can be applied successfully to older fingermarks.

This study further bolsters the potential of analytical methods such as GC–MS to provide an accurate account of chemical changes associated with fingermark degradation over time. While the rapid decrease in the relative concentration of squalene when exposed to light and airflow presents a potential method for monitoring fingermark age, it also makes this compound unsuitable as a target for fingermark detection. Other components such as free fatty acids and wax esters may be better suited due to their persistence in samples up to 28 days old, regardless of light exposure. Longer-term investigations are required to establish the relative degradation rates of these compounds, in order to narrow the selection of candidate targets based on their durability. Similar studies targeted towards a larger range of constituents, such as non-volatile di- and triglycerides, as well as degradation products, will enable a more detailed understanding of the processes that affect fingermark detection.

Such information will greatly assist in the development of novel methods for the detection of older fingermarks.

Acknowledgements

7. References

1. Mountfort, K.A., et al., *Identification of oxidation products of squalene in solution and in latent fingerprints by ESI-MS and LC/APCI-MS.* Analytical Chemistry, 2007. **79**(7): p. 2650-2657.
2. Koenig, A., A. Girod, and C. Weyermann, *Identification of wax esters in latent print residues by gas chromatography-mass spectrometry and their potential use as ageing parameters.* Journal of Forensic Identification, 2011. **61**(6): p. 652-676.
3. Midkiff, C.R., *Lifetime of a latent print: How long? Can you tell?* Journal of Forensic Identification, 1993. **43**(4): p. 386-392.
4. Weyermann, C., C. Roux, and C. Champod, *Initial results on the composition of fingerprints and its evolution as a function of time by GC/MS analysis.* Journal of Forensic Sciences, 2011. **56**(1): p. 102-108.
5. Dikshitulu, Y.S., et al., *Aging studies on fingerprint residues using thin-layer chromatography and high performance liquid chromatography.* Forensic Science International, 1986. **31**(4): p. 261-266.
6. Wertheim, K., *Fingerprint age determination: is there any hope?* Journal of Forensic Identification, 2003. **53**(1): p. 42-49.
7. Archer, N.E., et al., *Changes in the lipid composition of latent fingerprint residue with time after deposition on a surface.* Forensic Science International, 2005. **154**(2-3): p. 224-239.
8. De Paoli, G., et al., *Photo- and thermal-degradation studies of selected eccrine fingerprint constituents.* Journal of Forensic Sciences, 2010. **55**(4): p. 962-969.
9. Mong, G.M., C.E. Petersen, and T.R.W. Clauss, *Advanced Fingerprint Analysis Project: Fingerprint Constituents*. 1999, Pacific Northwest National Laboratory: Richland.
10. Richmond-Aylor, A., et al., *Thermal degradation analysis of amino acids in fingerprint residue by pyrolysis GC-MS to develop new latent fingerprint developing reagents.* Journal of Forensic Sciences, 2007. **52**(2): p. 380-382.
11. Baniuk, K., *Determination of age of fingerprints.* Forensic Science International, 1990. **46**(1-2): p. 133-137.
12. De Alcaraz-Fossoul, J., et al., *Determination of latent fingerprint degradation patterns - a real fieldwork study.* International Journal of Legal Medicine, 2013. **127**(4): p. 857-870.
13. Sutton, R., C. Grenci, and L. Hrubesova, *A comparison on the longevity of submerged marks in field and laboratory conditions.* Journal of Forensic Identification, 2014. **64**(2): p. 143-156.
14. Antoine, K.M., et al., *Chemical differences are observed in children's versus adults' latent fingerprints as a function of time.* Journal of Forensic Sciences, 2010. **55**(2): p. 513-518.
15. Buchanan, M.V., K. Asano, and A. Bohanon, *Chemical characterisation of fingerprints from adults and children*, in *Forensic Evidence Analysis and Crime Scene Investigation*. 1997, SPIE (International Society for Optical Engineering): Boston. p. 89-95.
16. Frick, A.A., G.E. Chidlow, and S.W. Lewis, *Investigations into the initial composition of latent fingermark lipids by gas chromatography-mass spectrometry.* Forensic Science International, 2015. **254**: p. 133-147.

17.   IFRG, *Guidelines for the assessment of fingermark detection techniques.* Journal of Forensic Identification, 2014. **64**(2): p. 174-200.

18.   Kent, T., *Standardizing protocols for fingerprint reagent testing.* Journal of Forensic Identification, 2010. **60**(3): p. 371-379.

19.   Lennard, C., *Fingerprint detection: current capabilities.* Australian Journal of Forensic Sciences, 2007. **39**(2): p. 55-71.

20.   Ramotowski, R., *Composition of Latent Print Residue*, in *Advances in Fingerprint Technology*, H. Lee and R. Gaensslen, Editors. 2001, CRC Press: Boca Raton. p. 63-104.

21.   Ramotowski, R., *Lipid Reagents*, in *Lee and Gaensslen's Advances in Fingerprint Technology*, R. Ramotowski, Editor. 2012, CRC Press: Boca Raton. p. 83-96.

22.   Salama, J., et al., *Evaluation of the Fingermark Reagent Oil Red O as a Possible Replacement for Physical Developer.* Journal of Forensic Identification, 2008. **58**(2): p. 203-237.

23.   Frick, A.A., et al., *A modified Oil Red O reagent for the detection of latent fingermarks on porous substrates.* Journal of Forensic Identification, 2012. **62**(6): p. 623-641.

24.   Bramble, S. and J. Brennan, *FINGERPRINTS (DACTYLOSCOPY): Chemistry of Print Residue*, in *Encyclopedia of Forensic Sciences*, J. Siegel, P. Saukko, and G. Knupfer, Editors. 2000, Academic Press: San Diego. p. 890-900.

25.   Girod, A., C. Roux, and C. Weyermann, *La datation des traces digitales (partie II): proposition d'une approche formelle.* Revue Internationale de Criminologie et Police Technique et Scientifique, 2014. **67**(2): p. 226-249.

26.   Jones, N.E., et al., *A systematic approach to latent fingerprint sample preparation for comparative chemical studies.* Journal of Forensic Identification, 2001. **52**(5): p. 504-515.

27.   Wargacki, S.P., L.A. Lewis, and M.D. Dadmun, *Enhancing the quality of aged latent fingerprints developed by superglue fuming: Loss and replenishment of initiator.* Journal of Forensic Sciences, 2008. **53**(5): p. 1138-1144.

28.   Williams, D.K., C.J. Brown, and J. Bruker, *Characterization of children's latent fingerprint residues by infrared microspectroscopy: Forensic implications.* Forensic Science International, 2011. **206**: p. 161-165.

29.   Wolstenholme, R., et al., *Study of latent fingermarks by matrix-assisted laser desorption/ionisation mass spectrometry imaging of endogenous lipids.* Rapid Communications in Mass Spectrometry, 2009. **23**(19): p. 3031-3039.

30.   Sauzier, G., et al., *Preliminary studies into the effect of environmental degradation on the characterisation of automotive clear coats by attenuated total reflectance infrared spectroscopy.* Analytical Methods, 2013. **5**(19): p. 4984-4990.

31.   Girod, A. and C. Weyermann, *Lipid composition of fingermark residue and donor classification using GC/MS.* Forensic Science International, 2014. **238**: p. 68-82.

32.   Croxton, R.S., et al., *Variation in amino acid and lipid composition of latent fingerprints.* Forensic Science International, 2010. **199**(1-3): p. 93-102.

33.   Miller, J.N. and J.C. Miller, *Statistics and Chemometrics for Analytical Chemistry*. 6th ed. 2010, Harlow: Prentice Hall.

34.   Brereton, R.G., *Applied Chemometrics for Scientists*. 2007, Chichester, England: John Wiley & Sons Ltd.

35.   Tan, B., J.K. Hardy, and R.E. Snavely, *Accelerant classification by gas chromatography/mass spectrometry and multivariate pattern recognition.* Analytica Chimica Acta, 2000. **6**(1): p. 37–46.

36.   Zamir, A., E. Springer, and B. Glattstein, *Fingerprints and DNA: STR typing of DNA extracted from adhesive tape after processing for fingerprints.* Journal of Forensic Sciences, 2000. **45**(3): p. 687-688.

37.   Adam, C.D., S.L. Sherratt, and V.L. Zholobenko, *Classification and individualisation of black ballpoint pen inks using principal component analysis of UV–vis absorption spectra.* Forensic Science International, 2008. **174**(1): p. 16-25.

499   38.   Thanasoulias, N.C., N.A. Parisis, and N.P. Evmiridis, *Multivariate chemometrics for the*
500   *forensic discrimination of blue ball-point pen inks based on their Vis spectra.* Forensic Science
501   International, 2003. **138**(1-3): p. 75-84.
502   39.   Mendlein, A., C. Szkudlarek, and J.V. Goodpaster, *Chemometrics*, in *Encyclopedia of Forensic*
503   *Sciences*, J.A. Siegel and P.J. Saukko, Editors. 2013, Academic Press: San Diego. p. 646-651.
504   40.   Zadora, G., *Chemometrics and Statistical Considerations in Forensic Science*, in *Encyclopedia*
505   *of Analytical Chemistry*, R.A. Myers, Editor. 2010, John Wiley & Sons: Chichester.
506   41.   Morgan, S.L. and E.G. Bartick, *Discrimination of Forensic Analytical Chemical Data Using*
507   *Multivariate Statistics*, in *Forensic Analysis on the Cutting Edge: New Methods for Trace*
508   *Evidence*, R.D. Blackledge, Editor. 2007, John Wiley & Sons: New Jersey. p. 333–374.
509   42.   Maric, M., W. van Bronswijk, and S.W. Lewis, *Rapid characterisation and classification of*
510   *automotive clear coats by attenuated total reflectance infrared spectroscopy.* Analytical
511   Methods, 2012. **4**(9): p. 2687-2693.
512   43.   Geladi, P., et al., *Chemometrics in spectroscopy Part 2. Examples.* Spectrochimica Acta Part B,
513   2004. **59**(9): p. 1347–1357.
514   44.   Ricci, C., et al., *Chemical imaging of latent fingerprint residues.* Applied Spectroscopy, 2007.
515   **61**(5): p. 514-522.
516   45.   Turner, D.A. and J.V. Goodpaster, *Comparing the effects of weathering and microbial*
517   *degradation on gasoline using principlal components analysis.* Journal of Forensic Sciences,
518   2012. **51**(1): p. 64-69.
519   46.   Yeo, H.C.H. and T. Shibamoto, *Formation of formaldehyde and malonaldehyde by*
520   *photooxidation of squalene.* Lipids, 1992. **27**(1): p. 50-53.
521   47.   van Dam, A., et al., *Oxidation monitoring by fluorescence spectroscopy reveals the age of*
522   *fingermarks.* Angewandte Chemie, 2014. **53**(24): p. 6272–6275.
523   48.   Barnett, P.D. and R.A. Berger, *The effects of temperature and humidity on the permamency*
524   *of latent fingerprints.* Journal of Forensic Sciences, 1977. **16**(3): p. 249-254.

525