Faculty of Science and Engineering
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Chemical Investigations into the Lipid Fraction of Latent Fingermark Residue

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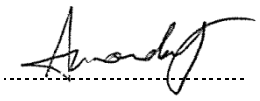
Declaration

To the best of my knowledge and belief this thesis contains no material previously published by any other person except where due acknowledgment has been made.

This thesis contains no material which has been accepted for the award of any other degree or diploma in any university.

Signature: 

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Abstract

The lipid fraction of latent fingermark residue encompasses a number of stable and durable compounds crucial to fingermark detection on wetted porous substrates. Additionally, the complex nature of fingermark lipids, and the chemical changes undergone by several of these compounds as a function of time, may provide a means to extract valuable information from a fingermark, such as its age, and characteristics of the individual to whom it belongs. This thesis describes a number of investigations focused on the detection and chemical analysis of the lipid fraction of latent fingermarks.

Two histological lipid stains were adapted for use as latent fingermark development reagents. It was found that Oil red O in propylene glycol yielded comparable results to a published Oil red O reagent, but has advantages in being less hazardous and simpler to prepare and use. The fatty acid stain Nile blue A was also investigated as a potential alternative to a novel Nile red reagent. It was found that the spontaneous hydrolysis of Nile blue A to Nile red in aqueous solution yielded a reagent that could develop latent fingermarks on a wide range of substrates, due to the presence of two lipid-sensitive compounds in one solution. The Nile blue reagent interacted with latent fingermarks on porous and some non-porous substrates to yield blue-purple impressions that, on several substrates, exhibit photoluminescence.

Latent fingermark samples were collected on white copy paper from 148 donors to compare the relative performance of Oil red O and physical developer on both recently deposited samples, and those stored for 30 days. Physical developer was found to outperform Oil red O on both fresh and stored samples, with Oil red O performance significantly worsening on older samples. Statistical methods revealed that donor age, sex and recent use of skin products had significant influence on physical developer performance on recently deposited samples. This variation appeared to decrease with increased sample age.

Latent fingermarks collected from 116 donors were analysed following solvent extraction. A simple gas chromatography-mass spectrometry method was developed for the detection of major fingermark lipid groups including free fatty acids, squalene, cholesterol and wax esters. Additional compounds were identified including components of cosmetic products and skin lotions, such as esters and vitamin E acetate. Principal component analysis was

used as an exploratory tool to understand variation in fingermark composition and changes in such that occur as a function of time. No correlation to donor traits could be discerned, and oftentimes significant intra-donor variation was observed. Changes in fingermark composition due to degradation were observed over a 4 week period. The rate of fingermark lipid degradation was shown to be donor-specific, and influenced markedly by storage conditions.

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Publications

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G. Sauzier, A.A. Frick, and S.W. Lewis, *Investigation into the performance of modified silver physical developers for visualizing latent fingermarks on paper.* Journal of Forensic Identification, 2013. **63**(1): p. 70-89.

A.A. Frick, P. Fritz, S.W. Lewis, W. van Bronswijk, *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.

Conference presentations

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Detection of latent fingermarks using a modified Oil Red O reagent. 21st International Symposium on the Forensic Sciences of the Australian and New Zealand Forensic Science Society, Hobart. September 2012.

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Abbreviations

AFP	Australian Federal Police
ATR	Attenuated total reflectance
DESI	Desorption electrospray ionisation
EDTA	Ethylenediaminetetraacetate
FID	Flame ionisation detection
FTIR	Fourier transform infrared
GC-MS	Gas chromatography-mass spectrometry
HOPSDB	Home Office Police Scientific Development Branch
IFRG	International Fingerprint Research Group
IR	Infrared
LDA	Linear discriminant analysis
MALDI	Matrix-assisted laser desorption ionisation
MS	Mass spectrometry
MSI	Mass spectrometry imaging
ORO	Oil red O
PC	Principal component
PCA	Principal component analysis
PD	Physical developer
SIMS	Secondary ion mass spectrometry
TIC	Total ion chromatogram
USSS	United States Secret Service

Chapter 1: Introduction

Portions of this chapter have been published in *Encyclopedia of Forensic Sciences* and *Journal of Forensic Identification*:

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1.1 Introduction

Fingermarks, the impressions left by contact between the skin ridges of the fingertips and a surface, are one of the most easily recognisable forms of identification evidence encountered by forensic investigators. Due to the unique nature of the patterns formed by these ridges, fingermarks have long been considered to provide conclusive proof of an individual's contact with the surface on which their fingermarks are found [1-3]. In this thesis, a distinction is made between fingermarks and fingerprints – 'fingerprints' are defined here as a deliberately made imprint of the skin ridges of the fingertip (i.e. for the purpose of database entry), while the term 'fingermarks' is used to refer to the imperfect (often smudged, distorted or incomplete) ridge impressions left by incidental contact [4, 5]. The most common type of fingermark found at crime scenes are latent or invisible fingermarks, which consist predominantly of substances secreted by the eccrine and sebaceous glands, as well as exogenous contaminants [5, 6]. Latent fingermarks are translucent, and so require chemical or physical treatments in order to be made visible for subsequent identification processes.

Though fingermarks have been used as identification evidence for over 100 years, relatively little is known about their composition, or how latent fingermarks interact with development reagents to produce a visible ridge pattern. Models of latent fingermark composition are based on medical knowledge pertaining to bulk skin secretions, and are not necessarily reflective of fingermark deposits [1, 3, 7, 8]. The age of a deposit can affect the efficacy of many latent fingermark development techniques, as some reagents react better with fresh fingermarks, while others perform increasingly well with an older fingermark [9]. However, there is currently little information on how latent fingermark residue degrades [9, 10]. In recent years, there has been increased interest towards addressing these issues, and this has proven to be a challenging task [10-15].

This chapter provides an overview of the composition of the lipid fraction of latent fingermarks and the lipid-sensitive chemical methods used to detect fingermarks on porous substrates.

1.2 Significance of fingermarks as forensic evidence

1.2.1 Friction ridge skin

The skin ridges present on the palms of the hands and the soles of the feet serve to assist grip by increasing friction. Many species of mammals, including primates and koalas, exhibit

ridge patterns similar to human fingerprint patterns [16]. The skin ridges of the fingertips, palms and feet develop over the 10^{th} to 16^{th} weeks of gestation in humans. The *in utero* formation of the skin ridges is not completely understood, though it is known to be influenced by many random factors [1, 17]. Genetics, timing of fingertip development processes, physical tension across the foetal skin, application of pressure and flexion of the developing skin all play a role in the development of both general ridge patterns and the minute ridge details that make fingermarks unique [18].

The use of these ridge patterns as identification evidence is accepted based on the following three principles: 1) Fingermarks are unique to an individual; 2) Fingermarks remain unchanged throughout the course of an individual's lifetime (with the exception of permanent scarring); 3) Fingermarks can be classified according to the patterns formed by the ridges [16, 18, 19]. Fingermarks have been used as identification evidence for over 100 years, yet identical fingermarks from more than one individual have never been recorded [16]. Even identical twins may be clearly distinguished from one another based on fingermark evidence, an area in which DNA profiling currently offers no such discriminatory value [16]. Fingermark patterns are not able to be altered or destroyed by superficial damage to the ridge skin; complete erasure of the ridges is often not possible in the long term, while the presence of scars only provides additional identifying details [18, 20].

1.2.2 Fingermark patterns and classification

The patterns and fine details formed by the skin ridges of the fingertips fall into one of three levels of detail, as proposed by Ashbaugh to simplify descriptions and comparisons [18, 21]. These levels of detail contribute differently to the examination of a fingermark, and consequent conclusions regarding its origin. Not all levels of detail may be seen in a single fingermark, depending on factors such as distortion and clarity that are reliant on deposition conditions [18].

Level 1 detail refers to the pattern of the fingermark and the general ridge flow of the palms and feet, which is readily observable without magnification [18, 21]. The systemic categorisation of fingermark patterns was originally developed in order to simplify the organisation of growing fingerprint databases [2, 20, 22]. Fingermark patterns fall under one of three broad categories: loops, whorls and arches (Figure 1.1). Loops are the most commonly occurring pattern, constituting approximately 65 % of fingermarks. Whorls comprise approximately 35 % of fingermarks, and arches make up the remaining 5 % [19]. There are several further subcategories of each of these three general patterns [2, 16, 20].

Level 1 detail does not offer enough discriminatory power to enable identification, but examination of ridge flow does assist in an initial narrowing down of the list of potential matches [18]. Regarding partial fingermarks, knowledge of level 1 detail can help determine the position and orientation of a fragment in the whole fingermark pattern, and thus assist in the comparison of finer ridge details.

Figure 1.1: Three main classes of fingerprint patterns;
loop (left), whorl (centre), and arch (right) [20]

Level 2 detail encompasses the small variations in ridge structure such as ridge endings and junctions, known as minutiae or Galton details, which require magnification (5 – 10x) for proper viewing [1]. The types and locations of minutiae within the fingermark pattern are what make each fingermark truly unique, rather than the overall ridge pattern, due to their seemingly random formation during skin ridge development [18]. Other distinguishing features on the skin ridges, such as warts, flexion creases and scars, also contribute to level 2 detail [16, 18, 23]. It is through careful examination of these small details that a fingermark can be matched to a single individual.

There is no standard minimum number of matching minutiae required for two fingermarks to be considered a conclusive match. Edmond Locard suggested that fewer than eight is not strong enough for a definite match. Eight to twelve matching points have often been used, though some countries have required as many as 16 [21]. In 1973, the International Association for Identification established that there was no scientific basis for the number of matching points needed for identification [18, 21]. This was further supported in 1995 by the signing of the Ne'urim Declaration at a conference in Israel, with unanimous agreement from representatives of 28 countries [1, 18]. It was thereafter recommended that the practice of matching a prescribed number of minutiae be discontinued in favour of a more flexible examination method of analysis, comparison, evaluation and verification (ACE-V) developed by the Royal Canadian Mounted Police [1, 18, 21]. Using this method, whether

two fingermarks are alike enough to be considered identical is left up to the discretion of the examiner, based on their own judgement and experience [2, 16, 18, 23].

Level 3 detail pertains to the details contained within a ridge: the shape of the ridge edge, and the location of eccrine pores along the ridge [18]. Higher magnification is required to examine these details than for level 2 details. The examination of level 3 detail, first proposed by Locard, is also referred to as poroscopy. It is often not as highly regarded as level 2 detail in identification comparison processes, as these features are often deposited inconsistently, making them less reliable than minutiae as identifying features [24]. Additionally, impressions of the pores generally occur only in very high quality fingermarks that exhibit little distortion or smudging [18].

1.2.3 Types of fingermarks

There are three categories of fingermarks that may be encountered in criminal investigations: plastic fingermarks, the ridge impressions left in malleable substances such as putty, clay or soap; patent or visible fingermarks, which are left on a surface by fingertips contaminated by a coloured substance such as ink or blood; and latent or invisible fingermarks, which consist of a translucent mixture of skin secretions (oils and sweat) as well as trace amounts of handled substances [2, 25]. This last type of fingermark must be treated so that the ridge pattern can be examined, and is the most commonly encountered in criminal investigations [3].

A surface examined for latent fingermarks is typically subjected to a series of physical and chemical development methods, ordered so that the success of each technique is not hindered by a preceding one [3]. The development methods utilised in such detection sequences are determined by surface type, which is broadly categorised into porous and non-porous, as well as whether the surface is dry, adhesive, or has been wetted [26]. While successful fingermark detection relies heavily on differences in chemical composition between the latent fingermark residue and its substrate [27], there are large gaps in the current understanding of latent fingermark composition. One consequence of this knowledge gap is that the mechanisms behind several of the more commonly used fingermark development reagents are yet to be fully characterised, which presents difficulties for the optimisation of these methods.

1.3 Latent fingermark composition

Latent fingermarks are a mixture of aqueous and lipid components [28, 29]. This residue is predominantly composed of secretions from the eccrine and sebaceous glands, as well as material from the epidermis and the apocrine glands [8, 30]. Hundreds of endogenous chemical species may be found in a single fingermark, including water, proteins, amino acids, lipids and salts. Trace amounts of exogenous contaminants, derived from the handling of substances such as food or grease, or from use of cosmetic products on the skin and hair, are also frequently present [5, 14, 25]. Some of these substances, such as illicit drugs and explosives residues, are highly important to criminal investigations [6, 31].

Current knowledge of latent fingermark composition is predominantly based on medical literature regarding skin secretions, rather than the unique mixture that is present on the fingertips [1, 3, 7, 8, 30]. Additionally, not all substances on a fingertip are transferred in a single contact; the mechanical transfer of substances from the fingertip to a surface is affected by factors such as fingertip pressure, duration of contact, surface type and temperature, which therefore may have an impact on fingermark composition [8, 28, 32]. For this reason, except when stated otherwise, the information below pertains to the composition of skin secretions rather than latent fingermarks.

1.3.1 Aqueous components

The water-soluble constituents of latent fingermarks are primarily sourced from the secretions of the eccrine glands. More commonly known as sweat glands, they are located all over the body, and are found in greatest density on the skin ridges of the palms of the hands and the soles of the feet. They are the sole type of secretory gland on the fingertips, therefore latent fingermarks generally contain some amount of eccrine sweat, if little else [1].

The eccrine glands and their secretory ducts form coiled, tubular structures within the dermis which open directly onto the skin surface [33]. Sweat is produced within the glands and packaged inside vesicles, to be released into the secretory ducts via exocytosis [33]. The main functions of eccrine sweat are to dissipate body heat through evaporation, improve grip on the hands and feet by moistening the skin, and to excrete excess water, electrolytes and waste products such as urea [16, 30].

Eccrine sweat is a primarily aqueous secretion consisting of salts and amino acids, as summarised in Table 1.1 [8, 34]. The exact composition of eccrine sweat varies greatly

between individuals and is known to be affected by variables such as health, diet and genetic factors [15, 35]. The rate of sweat production is influenced by stress, elevated environmental temperatures and physical activity [7, 35]. The response of the eccrine glands to elevated temperatures is gradual and weak, but is much stronger and more immediate when stimulated by stress, which is thought to be an important factor regarding the deposition of latent fingermarks at crime scenes [7].

Table 1.1: Summary of the composition of eccrine sweat [7]

Organic		**Inorganic (major)**		**Inorganic (trace)**
Amino acids	0.3 – 2.59 mg/L	Sodium	34 – 266 mEq/L	Magnesium
Proteins	15 – 25 mg/dL	Potassium	4.9 – 8.8 mEq/L	Zinc
Glucose	0.2 – 0.5 mg/dL	Calcium	3.4 mEq/L	Copper
Lactate	30 – 40 mM	Iron	1 – 70 mg/L	Cobalt
Urea	10 – 15 mM	Chloride	0.52 – 7 mg/L	Lead
Pyruvate	0.2 – 1.6 mM	Fluoride	0.2 – 1.18 mg/L	Manganese
Fatty acids	0.01 – 0.1 µg/mL	Bromide	0.2 – 0.5 mg/L	Molybdenum
Sterols	0.01 – 0.12 µg/mL	Iodide	5 – 12 µg/L	Tin
Creatine		Bicarbonate	15 – 20 mM	Mercury
Creatinine		Phosphate	10 – 17 mg/L	
Glycogen		Sulphate	7 – 190 mg/L	
Uric acid		Ammonia	0.5 – 8 mM	
Vitamins				

An infrequent and minor source of aqueous fingermark constituents are the apocrine glands. Apocrine glands are a type of sweat gland associated with hair shaft canals of the armpits and the groin [7]. Their function in humans is largely unknown, however they are thought to act as scent glands as they become functional at puberty, under the influence of androgens. Additionally, apocrine sweat develops a characteristic odour due to bacterial degradation on the surface of the skin [33].

Apocrine sweat is produced in granules, which are released from the cell together with small amounts of cytoplasm [33]. The secretions of the apocrine glands are otherwise largely similar to eccrine sweat (Table 1.2). Due to their location on the body, apocrine sweat is thought to rarely be a significant contributor to latent fingermark residue, except for cases of sexual assault [1].

Table 1.2: Summary of the composition of apocrine gland secretions [1]

Organic	Inorganic
Proteins	Water (>98 %)
Carbohydrates	Iron
Sterols	

1.3.2 Lipid components

1.3.2.1 Sources of fingermark lipids

The lipid material present on the surface of the skin is derived from three sources: the epidermis itself, and the secretions of the sebaceous and the eccrine glands [36]. Skin surface lipid composition is not uniform, as the relative contribution of sebaceous and epidermal lipids to total skin surface lipids will vary with anatomical location. Areas of skin rich in sebaceous glands will, unsurprisingly, contain a higher proportion of sebaceous lipids [37, 38]. The constant sloughing of epithelial cells from the stratum corneum, the topmost layer of the epidermis, is a major source of fingermark lipids in areas with low sebaceous gland activity [39-41]. Compositional differences between sebaceous and epidermal lipids are outlined in Table 1.3. Eccrine sweat does contain some lipid content, namely in the form of fatty acids and cholesterol, which are derived from the sloughing of epithelial cells within the secretory ducts, but this represents only a small fraction of latent fingermark lipids [42].

Table 1.3: Approximate composition of sebum and surface epidermal lipids [43]

Constituents	Sebum (wt %)	Surface epidermal lipid (wt %)
Glycerides plus free fatty acids	57.5	65
Wax esters	26.0	-
Squalene	12.0	-
Cholesterol esters	3.0	15
Cholesterol	1.5	20

1.3.2.2 Sebaceous lipids

The main source of fingermark lipids is sebum, an oily mixture produced by the sebaceous glands that contributes the majority (over 95 %) of lipid compounds found on the surface of human skin [39, 40, 43, 44]. Sebum is most commonly incorporated into fingermark residue

through the habitual touching of the face and scalp, often referred to as grooming motions [11, 15, 41, 44]. Epidermal lipids contribute a minor amount (3 – 6 %) to total skin surface lipids in these areas [37]. Therefore, as far as latent fingermarks are concerned, composition of the lipid fraction typically focuses solely on the sebaceous lipids. Sebum is estimated to contribute 5 % by weight of latent fingermark residue, however, the actual amount will naturally vary with the extent of grooming and removal of lipid material by washing [45].

The sebaceous glands consist of one or several lobes, encapsulated by highly vascularised connective tissue [46]. In humans, they are located almost all over the body, except for the palms of the hands and the soles of the feet, and are found in greatest density on the face and scalp. These glands are associated with hairs on the body, with the secretory ducts of these glands opening directly into the hair shaft canal [47]. The purpose of human sebum is largely unknown; its main functions are thought to include lubrication of the skin and hair, waterproofing of the epidermis, transportation of antioxidants and providing individuals with a unique scent signature [1, 47-52]. Most mammals and many birds produce sebum as a means of protecting feathers and fur from water, and to secrete pheromones and other chemical signals. Certain fatty acids also have antimicrobial properties, and are therefore thought to play a role in pathogen defence and the maintenance of skin homeostasis [40, 53, 54].

Sebum is produced within the sebaceous glands via a holocrine mechanism. Lipids are produced within maturing cells, and accumulate as these cells (sebocytes) continue to differentiate and migrate towards the centre of the gland [47, 55, 56]. During the final stage of differentiation, the mature sebocytes rupture and release their contents into the secretory ducts [7, 39, 46, 57]. The full process of lipid accumulation and secretion onto the skin surface is estimated to take 1 – 2 weeks [46, 48].

Sebum is a complex mixture of a number of lipidic compounds, consisting of hundreds of individual molecular species, including wax esters, triglycerides, free fatty acids, cholesterol and squalene [49]. Sebum composition is species-specific, with marked differences in the types and relative amounts of components observed even between closely-related species such as humans and chimpanzees [40, 46, 47, 58-60]. While many sebaceous components are produced in the sebaceous glands themselves, the presence of some essential fatty acids (such as linoleic acid) indicates that some of these lipids are exogenous in origin, and so are derived from the circulation rather than *de novo* synthesis in the sebaceous gland. As

with eccrine sweat, the composition of sebum can be affected by various factors such as genetics and diet [7].

The major compound classes present in human sebum and their relative abundances are listed in Table 1.4. The lipids present on the surface of human skin are markedly different to lipids synthesised within internal tissues [40, 55]. Due to a high proportion of unsaturated and branched chain compounds, the melting point of sebum is approximately 30 °C, allowing it to remain as a liquid film on the skin surface [55]. Additionally, it has been suggested that the abundance of unusual compounds and chain structures in sebaceous lipids may be a defence mechanism, as these lipids cannot be digested by bacterial enzymes and so inhibit pathogen growth [40, 59]. There is evidence to suggest that that hydrocarbons are exogenous contaminants, as labelled species were not detected in radiolabelling experiments [43].

Table 1.4: Percentage composition of the lipid classes within human sebum [55]

Lipid class	Percentage
Triglycerides	30 – 50
Free fatty acids	15 – 30
Wax esters	12 – 16
Squalene	10 – 12
Monoglycerides and diglycerides	5 – 10
Cholesterol	1 – 3
Cholesteryl esters	1 – 3
Hydrocarbons	1 – 3

Free fatty acids

Free fatty acids (carboxylic acids) are not secreted by the sebaceous glands, but are produced within the secretory ducts and on the skin surface as epidermal and bacterial lipases hydrolyse triglycerides to fatty acids, diglycerides, monoglycerides and glycerol [39, 52, 53, 61-65]. Free fatty acids make up approximately 15 – 25 % of sebum, though this proportion may vary significantly between individuals, or over time in a single individual, depending upon the extent of triglyceride hydrolysis [7, 14, 64, 66, 67]. Sebum is estimated to contain some 200 different fatty acid species, secreted in the form of triglycerides and wax esters [40].

Sebaceous fatty acids are unusual in that they display a wider range of structural variations than those found in other tissues, including many which are rare or only present in very low concentrations elsewhere [55]. Unusually long-chain fatty acids, up to 30 carbon units in length, have been reported in human sebum, as well as volatile short-chain fatty acids (< C10), which are thought to contribute to body odour [40]. The diversity of free fatty acids in sebum includes chains with an odd number of carbons, as well as methylated, hydroxylated, straight- and branched-chain structures [40, 54, 58, 68]. The relative proportions of these structures varies between individuals [67].

Saturated fatty acids, predominantly myristic (C14:0) and palmitic acid (C16:0; Figure 1.2), make up approximately 50 % of free fatty acids in sebum [7, 55]. Monounsaturated fatty acids comprise 48 % of sebum fatty acids, with straight-chain acids of 14 – 18 carbon units being dominant [54]. Uniquely, many unsaturated fatty acid species in human sebum have a double bond at the Δ6 position, or are derived from such, whereas monounsaturated fatty acids produced in other tissues (including in other species) typically have a double bond at the Δ9 position [40, 58, 69, 70]. The enzyme Δ6-desaturase is responsible for the production of these unusual fatty acids in the sebaceous glands; Δ9-desaturase is not expressed in this tissue [47, 71, 72]. This feature allows the differentiation between fatty acids synthesised in the sebaceous glands, and those sourced from the epidermis or incorporated into sebum from circulation, including those obtained from the diet (e.g. essential fatty acids).

The most abundant free fatty acid in human sebum is sapienic acid (C16:1Δ6; Figure 1.2), which as its name suggests, is unique to humans within the animal kingdom [58, 72, 73]. Sapienic acid, together with lauric acid (C12:0), are thought to possess antibacterial properties [49, 62]. The remaining 2 % of fatty acids in sebum are dienoic, with sebaleic acid (C18:2Δ5,8) and linoleic acid (C18:2Δ9,12) being the most abundant species [73]. Sebaleic acid is a 2 carbon extension product of sapienic acid, while linoleic acid is an essential fatty acid that plays a role in the maintenance of skin and hair health [58]. Trace amounts of other polyunsaturated fatty acids have also been reported [70]. The four most abundant free fatty acids reported in latent fingermark residue are oleic acid (C18:1Δ9), stearic acid (C18:0), palmitoleic acid (C16:1Δ9) and palmitic acid (C16:0) [10, 11, 49].

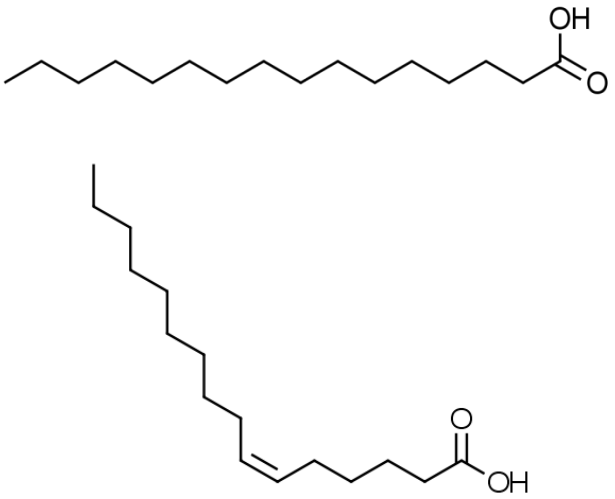

Figure 1.2: Molecular structures of palmitic acid (top) and sapienic acid (bottom)

Squalene, cholesterol and sterol esters

Squalene ($C_{30}H_{50}$; Figure 1.3) is an intermediate product formed in the biosynthesis of cholesterol, which is in turn a precursor in steroid hormone production [11, 44]. Squalene is produced in all tissues, but is usually cyclised to lanosterol. It is only in the skin that squalene reaches significant concentrations, though the reason for this is unknown [40, 72]. It has been suggested that squalene accumulates in the sebaceous glands due to substrate competition for the enzyme cofactor nicotinamide adenine dinucleotide phosphate, which is essential for cholesterol production, or simply due to low activity of the responsible enzymes [74].

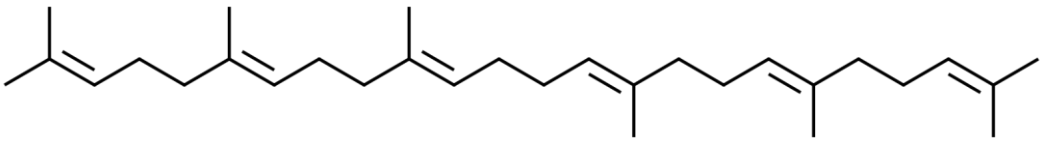

Figure 1.3: Molecular structure of squalene

As squalene is not rapidly converted to lanosterol in the sebaceous glands, it is thought that most of the cholesterol (Figure 1.4) in skin surface lipids is of epidermal origin, produced during differentiation of the epithelial cells of the epidermis [44, 49, 75]. This is further evidenced by the fact that squalene levels are higher in lipid samples from areas of skin rich in sebaceous glands, while cholesterol is higher in areas containing fewer sebaceous glands [43, 49]. The squalene : cholesterol ratio of skin lipids is thus used as a measure of sebaceous gland activity [49, 76]. Similarly, cholesteryl esters (2 – 3 %), the predominant sterol esters in human sebum [77], are thought to arise from esterification of epidermal

cholesterol with sebaceous fatty acids by bacteria on the skin surface, rather than from sebaceous gland activity [78-81]. Unsaturated fatty acids predominate over saturated fatty acids in these esters [82]. Fatty acid lengths of 14 – 18 carbon units have been reported [49].

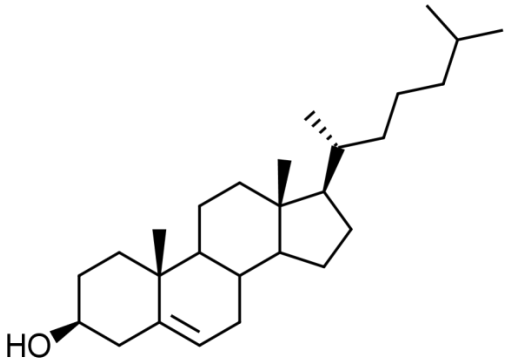

Figure 1.4: Molecular structure of cholesterol

Wax esters

Wax esters (Figure 1.5) are produced exclusively in the sebaceous glands, from the esterification of a long-chain fatty acid to a fatty alcohol [47, 58, 83]. Although wax esters are produced in many plant and animal species, for purposes such as surface protection and energy storage, their function in humans is not understood [84, 85]. The analysis of wax esters has often involved hydrolysis to their constituent fatty acids and alcohols, and so many wax ester structures have yet to be characterised in detail (i.e. the specific combinations of fatty acids to fatty alcohols and branch positioning) [14, 50, 68, 86].

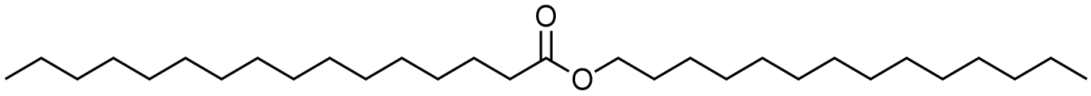

Figure 1.5: Molecular structure of myristyl palmitate

Over 200 wax esters have been detected in sebum extracted from a sample of human hair, ranging from 24 to over 40 total carbon units in size [49, 68, 84]. The predominant structures are straight-chained, and include saturated fatty acids esterified to saturated fatty alcohols, and monounsaturated fatty acids esterified to a saturated fatty alcohol [82, 87]. Wax esters may also contain branched-chain saturated and unsaturated fatty acids that contain methyl groups at either the iso (penultimate carbon) or anteiso (third carbon from the end) positions, however methyl groups at other positions are also present in saturated fatty acids [67, 85]. Di- and polyenoic wax esters have also been identified [40, 81].

The fatty acid profiles of wax esters have been found to vary significantly between individuals [67]. Major fatty acids of wax esters include myristic, palmitic and stearic acid [84, 88]. It has been suggested that genetic control may be responsible for this variation, as these profiles remain relatively constant over at least a short period of time, and so are unlikely to be affected by external factors such as diet [67, 69].

As with the fatty acids, fatty alcohols encompass a broad range of structures, including straight- and branched-chained, odd-numbered, saturated and unsaturated forms [88-90]. They are produced by the reduction of free fatty acids formed in the sebaceous glands; many unsaturated fatty alcohols contain a double bond at the Δ6 position, indicating a shared biosynthetic origin with the sebaceous fatty acids [83]. Free fatty alcohols are not present in appreciable amounts in sebum, presumably because wax esters are not easily hydrolysed by either bacterial or epidermal enzymes [7, 49, 55, 64, 69]. Fatty alcohols with chain lengths ranging from 14 – 27 carbon units have been reported, with even number chain structures predominating [49, 90]. Eicosanyl alcohol (C20:0) and its monounsaturate have been identified as the predominant fatty alcohol structures in human wax esters [83, 88].

Triglycerides

The identities of the sebaceous triglycerides (Figure 1.6) are largely unknown, as most studies into sebum composition have involved hydrolysing triglycerides into their constituent fatty acids, rather than the direct characterisation of the triglycerides themselves [4, 50, 91]. Although this has simplified research of the total fatty acid fraction of sebum, the method destroys structural information regarding the arrangement of these fatty acids into triglycerides [4].

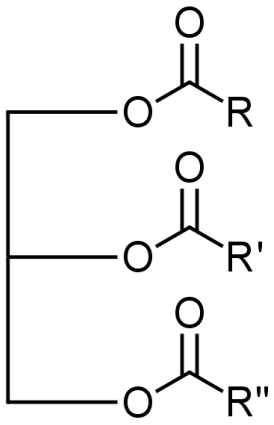

Figure 1.6: Molecular structure of a triglyceride

Saturated fatty acids are predominant in sebaceous triglycerides, while the relative proportions of structure types such as straight- and branched-chain acids fluctuates with age [82]. Triglycerides sampled from the skin surface contain fatty acids originating from the epidermis and the sebaceous glands, indicating two different sources of triglycerides in skin surface lipids [91, 92]. Radiolabelling experiments suggest that sebaceous triglycerides might instead be synthesised in the sebaceous glands from a combination of sebaceous fatty acids and fatty acids derived from circulating lipids in the plasma [58].

1.3.3 Factors affecting skin surface lipid composition

1.3.3.1 Physical maturation and age

The production of sebum is largely under the control of androgenic hormones [92]. Testosterone in particular has a potent effect upon the size of the sebaceous glands, which has a direct impact upon production rate [57, 93, 94]. Only small amounts of these hormones are required to produce significant gland enlargement, which is an important factor in skin pathology. Trace amounts of androgen metabolites have been reported in sebum collected from adult males [95]. Plasma testosterone concentration cannot be directly correlated to sebum production, however, indicating that factors other than androgen stimulus may be involved. Excessive levels of oestrogens, for example, appear to suppress sebum production, and are used as a treatment for acne [92, 93, 96]. Though the exact mechanism is unclear, it is thought that elevated concentrations of oestrogens antagonise androgen production [47, 57]. Changes in androgen levels that occur with age therefore impact upon sebum production over the course of an individual's lifetime. Significant changes in the quantity and composition of skin surface lipids with age and sex are well documented, with similar observations made in latent fingermark investigations [10, 11, 82, 97, 98].

During the last trimester of gestation, the foetus' skin becomes covered in a protective, waxy layer known as vernix caseosa, a mixture of sebaceous and epidermal lipids [48, 79]. Vernix caseosa is thought to be produced by the sebaceous glands under the influence of maternal androgens transferred to the foetus through the placenta. This substance is closely similar in composition to sebum [99]. However, vernix caseosa contains a larger proportion of Δ9 unsaturated lipids and sterol esters, and lacks free fatty acids, due to the absence of skin flora in the *in utero* environment [46, 79]. Male foetuses and newborns produce more lipid material than females, suggesting that sebum production is partly stimulated by the foetus' own androgens [82]. In the weeks following birth, lipid production

gradually ceases, and the sebaceous glands decrease in size, remaining largely inactive until the onset of puberty [61, 100].

Prepubescent children produce little sebum, and therefore have a markedly different skin surface lipid profile when compared to adults, with the majority of children's skin surface lipids being of epidermal origin. Additionally, the sebum of young children is markedly different to that produced by adults [48, 100, 101]. Samples taken from subjects in this age range contain a higher proportion of straight-chain fatty acids, as well as cholesterol, sterol esters and other epidermal lipids, and a lower proportion of wax esters [38, 44, 69, 75, 98, 100, 101]. Very young children's fingermarks have often been found to contain little or no squalene or fatty acids, or to be comprised mainly of volatile lipids, which accounts for the rapid evaporation of children's fingermarks following deposition [11, 44, 102]. This can create complications regarding the investigation of crimes involving children [44, 102]. Sebum similar in composition to that of adults' has also been observed in children nearing puberty [11, 100, 103]. This change in composition is thought to be caused by the onset of adrenarche, an early stage of sexual development which occurs at approximately 7 – 10 years of age, before any outward sign of maturation [48, 93, 94, 100]. It has been reported that skin surface cholesterol reaches maximal levels in some adolescents, particularly in females [11, 75]. The increased proportion of this compound may be due to an increase in mitotic activity in the skin, which is under the control of oestrogens and androgens in females and males respectively [75]. As sebaceous gland activity increases, and sebaceous lipids constitute the majority of skin surface lipids, cholesterol becomes a minor component.

During early adolescence (11 – 15 years old), female subjects typically produce more sebum than males, due to the earlier age at which puberty commences in females [75, 104]. Females in this age group who have not yet begun menstruation secrete significantly less sebum. As puberty progresses, the relative proportions of sebaceous lipids such as wax esters and squalene increases [61]. Similarly, relative proportions of fatty acid types in wax esters, sterol esters and triglycerides also change. A greater proportion of fatty acids incorporated into these lipids are of sebaceous origin, rather than derived from circulation [61].

Maximal rates of wax ester and squalene production (indicative of high sebaceous gland activity) occur throughout puberty and early adulthood. Sebum production remains at relatively stable levels during this time, after which a gradual decline in the proportions of

these substances is observed, beginning around the age of 40 [75, 105]. As well as the relative concentrations of sebaceous lipids, the dominant structural types of fatty acids changes with age. In triglycerides, saturated straight-chained fatty acids become more predominant with advancing age until senescence [82].

Towards senescence, further changes in androgen levels impact skin surface lipid composition. It has been suggested that the decline in sebaceous gland activity is due to a decreased sensitivity of the sebaceous glands to androgens, as well as the concentration of circulating androgens [104, 105]. Sebum production subsequently decreases, with a more rapid decrease that occurs at an earlier age in females (linked to the onset of menopause) than males [82, 97, 104]. Sebum composition gradually changes, and the proportion of wax esters and squalene diminishes [106]. As a result, the relative proportions of cholesterol, sterol esters and Δ9 fatty acids increase once more, as epidermal lipids become the predominant source of skin surface lipids [69]. With time, sebum composition more closely resembles that of young children, although there appears to be much individual variation in this rate of decline, as some elderly individuals may still produce 'adult-like' sebum into old age [82, 105].

1.3.3.2 Biological sex

As sebaceous gland activity is under the influence of androgens, it follows that male and female subjects might exhibit differences in sebum secretion rate or composition [66]. Significant differences in sebum production have been demonstrated between male and female subjects around the onset of puberty, as discussed above [75]. During adulthood, males generally produce greater amounts of sebum than females, due to sex-related differences in hormone levels [82, 93, 97]. The sebum produced by adult females tends to contain a greater proportion of Δ9 fatty acids, and a lower proportion of wax esters than males, indicating lower sebaceous gland activity, and a greater percentage contribution of epidermal lipids to total skin surface lipids [75, 82].

To date, investigations into skin lipid or fingermark composition with adult subjects have found no significant differences related to biological sex [15, 41]. While some compositional differences have been observed, none of the results obtained in these studies were considered statistically significant [4, 41, 75].

1.3.3.3 Diet

Dietary influences also have an impact upon sebum production and composition, particularly with the onset of acne [71]. Sebum contains a number of essential fatty acids,

such as linoleic acid, which are introduced into the sebaceous glands from the surrounding plasma [96]. Additionally, the consumption of carbohydrates and total caloric intake are known to have significant effects upon skin surface lipids [71].

Low-calorie diets bring about a significant reduction in sebum production in both healthy and obese individuals [107, 108]. Additionally, individuals who have fasted for a period of several days to weeks show a higher proportion of squalene in their sebum, due to a significant reduction (approximately 40 %) of the secretion of all other sebaceous components, presumably due to inhibition of fatty acid synthesis [107, 108]. Squalene production itself remains unaffected [107]. Conversely, high-calorie diets are thought to contribute to excessive sebum production [71]. A low glycaemic load diet has been found to reduce the ratio of saturated : unsaturated triglyceride fatty acids, indicating that the desaturase enzyme responsible for producing unsaturated fatty acids is affected [71].

Other types of diet (i.e. vegetarianism) may also affect skin lipid composition [15, 109]. A study into volatile skin compounds by Gallagher *et al*. indicated that frequent consumption of seafood might lead to increased concentrations of unsaturated aldehydes, which contribute to body odour [52].

1.3.3.4 Intra-individual variation

It has been proposed that individual traits such as those discussed above may be inferred from fingermark composition, such that characterisation of fingermarks can be used in identification down to an individual level [11, 44]. For such an approach to be valid, it is essential to first establish whether or not an individual's fingermark composition remains consistent over an extended period of time. While there are many studies that aim to determine if significant differences can be observed in latent fingermark composition, such information is useless if individuals' skin surface lipid composition fluctuates significantly over time.

Several studies suggest that the skin surface lipid composition of an individual may be subject to natural quantitative and qualitative variation. Such studies have produced mixed results. Squalene concentration in sebum collected from subjects' backs has been shown to vary over the course of a month, while cholesterol levels remained relatively constant [38]. Similarly, intra-donor variation in sebum collected from the chest over 7 weeks has been observed [66]. Conversely, it has been reported that that skin surface lipids from the forehead remained relatively consistent in composition over both short- and long-term (14 months) periods [64]. Monitoring of sebum composition found no fluctuations in

composition that could be correlated to the menstrual cycle in adult female subjects [38, 75], but the rate of sebum secretion from forehead skin has been demonstrated to follow a circadian rhythm [110-112]. Analysis of volatile compounds emanated from the skin surface indicates that metabolic changes correlated to seasonal variations may have some impact on composition [113], though it is unclear whether such trends may be exhibited by non-volatile sebaceous lipids. Disease states which alter metabolic processes may impact upon the composition of substances excreted onto the skin surface, such that the profiling of volatile biomarkers may be used as a non-invasive diagnostic tool [113-118].

The use of oral antibiotics, which would have a suppressive effect on bacterial lipolysis, may result in decreased free fatty acid content in sebum [15]. Topical antibiotic treatments appear to have little effect on triglyceride hydrolysis, presumably as the anaerobic bacteria responsible are located within the hair shaft canals and secretory ducts of the sebaceous glands, rather than the skin surface [65]. However, acne treatments have been found to affect fingermark lipid quantity through removal of excess sebum [14].

Preliminary results by Koenig *et al*. suggest that intra-donor variation may occur to a significant extent in latent fingermarks [14]. Asano *et al*. however were unable to identify any statistically significant changes in composition attributable to short-term variation [41]. There is additional evidence to suggest that fingermark composition varies with digit and handedness, however the significance of such variation currently remains unclear [119, 120].

1.3.4 Degradation of latent fingermark residue

Latent fingermark composition begins to be altered within a short period of time following deposition [41, 121]. This adversely affects the efficacy of many latent fingermark development techniques, which are most effective on fingermarks which are less than a few weeks old [7, 10, 122]. There is currently little information on how latent fingermark residue ages, or how it is impacted upon by environmental factors, bacterial activity or possibly even the application of development reagents [8, 10, 14, 122, 123]. Environmental conditions, including light exposure, substrate type, temperature, humidity, airflow and immersion in water, are thought to play a significant role in degradation rate; however, little is known about their specific impacts upon fingermark chemistry [5, 13, 45, 103, 121, 124]. Studies into the ageing of latent fingermarks are complicated by difficulties in obtaining homogenous samples, the natural variability between fingermark donors, and

exogenous contamination, such that a timeframe for latent fingermark degradation processes is difficult to establish [11, 123, 125].

The initial composition of a latent fingermark has great influence on its longevity [10, 13, 121]. The clearest example of this is young children's fingermarks, which contain very little non-volatile lipid material, and so degrade differently over time compared to adults' [98]. Depending on environmental conditions, one of the first degradation processes that occurs in latent fingermarks is a significant loss of mass, primarily though the evaporation of water, within an hour of deposition [120]. Further losses of other volatile compounds continue over a longer period, such that up to 85 % of a fingermark may be lost to volatilisation within two weeks following deposition [11]. The remaining residue is a brittle, waxy substance composed of salts and non-volatile lipids, that on non-porous surfaces is subject to erosion [10, 11, 13, 45, 121]. As such, older fingermarks are less amenable to visualisation with powders, which rely on the mechanical adherence of particles to lipids and moisture, and lipophilic dyes, which partition most readily into liquefied lipids [11]. As children's fingermarks contain a lower proportion of lipids, and less material in general, powdering is a less successful method of detecting children's fingermarks [98]. When exposed to elevated temperatures, children's fingermarks may evaporate within as little as 24 hours, contributing to the observation that children's fingermarks 'disappear' faster than adults' [98, 102].

As squalene is a highly unsaturated compound, it readily photooxidises to hydroperoxides and squalene epoxide, and eventually degrades completely to volatile compounds such as aldehydes and acetone, which are then lost to evaporation [122, 126]. It has also been suggested that squalene undergoes polymerisation [10, 11]. The effects of storage conditions on the rate of squalene degradation were investigated by Archer *et al*. [10]. It was found that when fingermark samples were stored with constant exposure to light, squalene degraded rapidly within the first week following deposition, and neither squalene nor its degradation products were detectable after this period [122]. When fingermarks were stored in complete darkness, the rate of oxidation was found to be much slower and squalene was still detected in fingermarks up to approximately one month after deposition [10, 13]. Mong *et al*. reported that squalene was not detected from fingermarks wrapped in foil [11], indicating that other factors may influence the degradation rate of squalene in addition to light exposure.

Degradation processes have also been observed with free fatty acids, wax esters and possibly triglycerides. Archer *et al*. proposed that observed changes in fatty acid levels might be due to bacterial degradation of wax esters and triglycerides to free fatty acids, which in turn are further degraded by oxidation or bacterial mechanisms or evaporate [10]. Unsaturated free fatty acids and wax esters appear to degrade more rapidly than their saturated counterparts, although at a slower rate than squalene, and are thought to become split at the double bonds [11].

1.3.5 Analytical studies of latent fingermark composition

The interaction that occurs between a latent fingermark deposit and a development reagent is directly dependent upon chemical composition. A more detailed understanding of fingermark chemistry, particularly the effects of degradation processes, is vital in order to develop new, more effective development methods, as well as to optimise existing ones. There has been increased interest in recent years into obtaining a greater understanding of latent fingermark composition towards these goals [5, 10, 11]. For the most part, such research has focused on the groups of compounds currently most pertinent to latent fingermark detection, i.e. amino acids and lipids.

A more complete understanding of latent fingermark composition may enable fingermark evidence to provide more information than just the ridge details. It is thought that traits such as age, sex or ethnic background could be inferred from fingermark composition [103, 127]. Such information would be of significant assistance to criminal investigations, if a fingermark found at a crime scene could not be matched to any in a database, or if the clarity of the ridge pattern was in some way unsuitable for comparative purposes [127-129]. As fingermark composition changes with degradation, it has also been proposed that a method for estimating the age of a fingermark could be developed, as a means of supporting or discrediting a testimony [4, 13].

1.3.5.1 Early studies of fingermark composition

Latent fingermark detection was initially carried out with little detailed knowledge about actual fingermark composition. Initial models appear to have been based on eccrine sweat as a major component, with possible contribution from sebaceous, apocrine or exogenous sources [130]. Further speculation was made based on the interaction of latent fingermarks with reagents such as ninhydrin, which was known to react with free amino acids [130].

A series of studies beginning in the 1960s at the UK Atomic Weapons Research Establishment provided a more in-depth analysis of fingermark components, with a specific focus on the water-soluble fraction; i.e. amino acids and chloride [119, 130, 131]. It was found that chloride concentration in fingermark deposits varied significantly with substrate type and donor age. Similar studies were conducted concerning the lipid fraction, confirming that fingermark lipids were derived from contact between the fingertips and lipid-rich skin such as the scalp and forehead [132]. These studies demonstrated that fingermarks deposited from recently cleaned fingertips yielded little lipid content, and that lipids did not replenish directly on fingertips, as ridge skin does not contain any sebaceous glands. Furthermore, it was found that the use of skin products and cosmetics, particularly in the case of female donors, introduced a number of extra compounds into fingermark samples [133].

1.3.5.2 Infrared spectroscopy

Infrared (IR) spectroscopy has the advantage of being non-destructive and requiring no sample preparation, making it suitable for use in conjunction with fingermark development methods [26, 31, 98, 134, 135]. Mid- and near-infrared Fourier transform IR (FTIR) and Raman spectroscopy have been utilised in a variety of investigations on the endogenous composition of latent fingermarks, as well as the detection of illicit drugs in fingermark residue [31, 136]. The improved resolution that can be achieved with IR microscopy enables examination of the heterogeneous nature of fingermark residue, and individual particles such as skin cell debris and sweat droplets [31, 98, 137].

FTIR spectroscopy has been used to examine differences between children's and adults' fingermarks [98, 103, 136, 137]. Additionally, the proportion of branched lipids in samples can be demonstrated based on the relative signals of CH_3 and CH_2 stretches [98]. This approach has been used to monitor both compositional differences and their influence on degradation processes [98]. In light of the impact that sebum composition has on the composition and durability of fingermark residue, an attempt has been conducted to correlate fingermark composition with donor age, using FTIR in combination with partial least squares regression [9]. Attenuated total reflectance (ATR) FTIR microscopy has been found to be ineffective in the analysis of eccrine latent fingermarks, which form a series of droplets on a non-porous substrate and have a detrimental effect on signal [137]. In these instances, better results may be obtained using reflection-absorption mode. ATR-FTIR can be used on lipid-rich fingermarks, as the higher lipid content results in continuous ridges [26].

The main drawback of IR techniques is their limited ability to analyse complicated mixtures [138]. IR methods lack the sensitivity and selectivity of mass spectrometry (MS) approaches, and are therefore less suited to detailed characterisation of complex mixtures such as latent fingermarks, which are comprised of hundreds of organic and inorganic species [129].

1.3.5.3 Chemical imaging

Chemical imaging is a variation on conventional MS and IR techniques whereby 2D images of a surface are constructed that exhibit the spatial distribution of surface components, as well as spectral information [139]. Spectra are acquired in a grid pattern and this data is converted into a 2D image, where each point of spectrum acquisition is seen as an individual pixel. This image can be viewed at any point within the analysed *m/z* or wavenumber range, to view signal distribution across the sample surface. Chemical imaging has several advantages over chromatography analyses, and so has potential for applications in fingermark composition research [128, 140].

Mass spectrometry imaging (MSI), FTIR and Raman imaging have been used to demonstrate the heterogeneous nature of fingermark residue, though often with a larger focus on visualising fingermark patterns rather than compositional analysis [5, 6, 26, 98, 141, 142]. These methods require little or no sample preparation, and are relatively non-destructive, as MSI techniques still leave a significant amount of fingermark residue intact, allowing for the degradation of fingermark compounds to be monitored, while IR is non-destructive. It has therefore been proposed that chemical imaging techniques may provide a complementary or alternative approach to conventional latent fingermark development, as these methods exploit the spectral differences between a fingermark and its substrate, and are less likely to be affected by a substrate's colour or photoluminescent properties [135, 141, 143-147]. Additionally, overlapping prints may be readily distinguished based on significant compositional differences [6, 145, 146]. Further information may be gleaned from latent fingermarks in addition to ridge pattern, through the detection of exogenous contaminants such as drugs, explosives and fibres [6, 148].

IR imaging has been explored primarily in fingermark detection, rather than direct compositional analysis. While FTIR microscopy is not as sensitive a technique as gas chromatography-mass spectrometry (GC-MS), it has the advantages of being non-destructive, and is not limited by compound solubility [103, 149]. The main disadvantages

of FTIR imaging techniques are the same as those of conventional IR spectroscopy, as described above.

MSI has poorer spatial resolution than IR imaging, but has the advantage of greater specificity. Ionisation techniques that have been adapted to MSI include matrix-assisted laser desorption ionisation (MALDI), desorption electrospray ionisation (DESI) and secondary ion mass spectrometry (SIMS), all of which have been applied to latent fingermarks. MALDI-MSI studies of latent fingermarks have focused mainly on lipid components, although the detection of amino acids and exogenous compounds has also been demonstrated [5, 129, 150, 151]. SIMS imaging of latent fingermarks has been used primarily to detect exogenous contaminants such as amphetamines and gunshot residue [152, 153]. DESI-MSI has been applied to the imaging of exogenous contaminants in fingermarks, such as illicit drugs and explosive residues, as well as endogenous compounds such as fatty acids and triglycerides [6].

While there is clear potential for MSI in the analysis of latent fingermark composition, it must be noted that these methods, particularly MALDI- and DESI-MSI, are relatively new and have mainly been applied to whole tissue samples in biomedical research. Even in this area, MSI is still regarded as being at an experimental stage of development [154-156]. One of the major issues still to be addressed is the lack of standard protocols for routine analyses; sample preparation and instrument settings are fine-tuned for every experiment. Regarding fingermarks, further work is needed to determine how many classes of compounds may be analysed simultaneously, and what sample preparation methods might improve current capabilities. It is also unclear whether or not the *m/z* distribution images produced by MSI are truly representative of relative component concentration. Thus far, there has been no reported comparison between MSI and chromatography-mass spectrometry techniques to determine if ion suppression effects produce significant interference [157].

1.3.5.4 Gas chromatography-mass spectrometry

GC was one of the first analytical techniques used to investigate latent fingermark composition, and GC-MS remains one of the most frequently utilised techniques for analysing latent fingermark lipids [25, 127]. The studies mentioned below are discussed in greater detail in Chapters 5 and 6.

A study was conducted by Buchanan *et al.* to investigate compositional differences between children's and adults' fingermarks [44, 102, 158]. Mong *et al*. reported the first

study into fingermark degradation processes, with the aim of identifying compounds for fingermark detection [11]. Both studies postulated that the observed compositional variation between donors might enable the identification of individual traits [44]. Initial investigations into differences due to gender were carried out by Asano *et al*. as a possible means of determining individual traits in circumstances where the pattern of a fingermark proved unsuitable for identification, however, no significant differences were observed [41].

Croxton *et al*. developed an approach to analyse both amino and fatty acids in latent fingermarks [32]. This method was used to establish that the practice of collecting deliberately sebum-rich (charged) fingermarks for analysis might cause some compounds to be overrepresented, with a greater impact on fatty acids than amino acids, such that the two fingermark types could be resolved using multivariate statistics [15]. Additionally, difficulties in differentiating samples based on donor traits were highlighted.

A different approach to fingermark degradation processes was taken by Weyermann *et al*. in their investigations towards developing a method of estimating fingermark age [13]. The sample preparation method used was similar to that of Asano *et al*. in that no derivatisation was used [41]. This method has enabled the identification of a number of lipid components, including wax esters [14]. A related study by Girod *et al*. found that individuals could be classified as lipid-rich and lipid-poor fingermark donors for research purposes [25].

1.4 Lipid-sensitive fingermark development reagents for porous substrates

Latent fingermark detection on porous substrates (i.e. paper) is predominantly carried out using amino acid-sensitive reagents such as ninhydrin, 1,2-indanedione and 1,8-diazafluoren-9-one [30, 159, 160]. These methods are highly sensitive, produce photoluminescent products (or those that can be treated with metal salts to produce photoluminescent complexes) and develop fingermarks rapidly. This provides a significant advantage when examining dark-coloured or patterned surfaces, on which a developed fingermark cannot be readily seen under conventional lighting. Due to the strong hydrogen bonding of amino acids to the cellulose fibres of paper, these reagents can be effective at developing fingermarks that are several decades old [1, 161]. As free amino acids are water-soluble, amino acid-sensitive reagents are less effective for latent fingermark detection on substrates which have been wetted or exposed to high humidity [30, 159]. On

wetted porous substrates, physical developer (PD) is typically used, although recently, lipophilic dyes have seen increased use.

1.4.1 Physical developer

Physical developer (PD) is a silver nitrate-based fingermark development method that is based upon a now-defunct photographic development process [29, 162-164]. It was often observed that the silver physical developer reagent left fingermark impressions on photographic plates that had been handled with bare hands [29]. The Atomic Weapons Research Establishment, working under the Police Scientific Development Branch of the UK Home Office, adapted the physical developer method for the detection of latent fingermarks on porous surfaces in the 1970s [1, 29]. This original PD formulation is what is referred to as the UK-PD formulation, currently in use by European and Australian forensic laboratories. The UK-PD formulation has remained essentially unchanged since its development in 1975 [164].

PD treatment involves the reduction of silver ions (Ag^{+}) to silver particles (Ag^{0}) in solution by a ferrous/ferric oxidation-reduction system, stabilised by the presence of citrate and a cationic surfactant (Figure 1.7). The selective accumulation of silver particles on fingermark residue allows the pattern of the ridges to be observed. PD interacts with the water-insoluble fraction of latent fingermark residue, making it one of the few development techniques that can be successfully utilised on porous surfaces that have been exposed to water or high humidity, conditions which wash away the target compounds of amino acid-sensitive reagents [1, 29]. The use of PD on porous surfaces following treatment with amino acid-sensitive reagents increases the number of fingermarks detected compared to amino acid-sensitive reagents alone [165]. Though PD has been shown to target the sebaceous lipid components of latent fingermark residue, evidence suggests that PD also interacts with some non-lipid compounds that become trapped within the hydrophobic lipid residue [1, 162, 166].

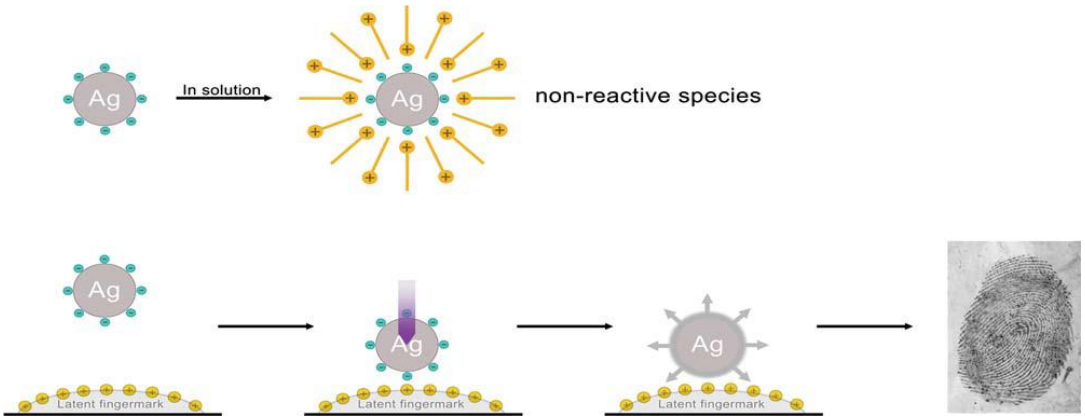

Figure 1.7: Schematic illustration of processes involved in the physical developer detection technique in solution (top) and close to fingermark residue (bottom) [167].

The exact mechanism for the interaction between the silver particles and fingermark residue is unclear; silver deposition will occur on the substrate as well as the fingermark under a variety of conditions, to produce dark grey fingermarks on a light grey background on white paper [165]. One widely accepted hypothesis for the interaction between PD and fingermarks is that the deposition of silver on latent fingermark residue is triggered by electrostatic attraction between the silver particles and certain fingermark components. These are thought to include unsaturated lipids, large, water-insoluble proteins, lipoproteins and possibly amino acids trapped within the residue [159, 164, 166, 167]. Amine functional groups become protonated in a low pH environment, such as that provided by citric acid [1, 164, 167]. It is thought that negatively charged silver particles formed sufficiently close to the fingermark reside become electrostatically attracted to the positively charged residue components, thereby forming nucleation sites for further aggregation of silver particles [11, 164].

The presence of citrate ions and a cationic surfactant (N-dodecylamine acetate) is intended to stabilise the working solution by preventing the rapid, uncontrolled formation of large silver particles, which results in poorer ridge development [168]. When silver ions are spontaneously reduced in solution, citrate ions become adsorbed onto the surface of the particles, conferring a negative charge. This attracts cationic surfactant molecules, thereby forming a protective layer that impedes particle growth, by both preventing the electrostatic attraction of silver ions and acting as a physical barrier. These particles are not thought to play a role in fingermark development [167]. PD working solutions that contain no surfactants have been devised, but they require a significant reduction in the

concentration of redox components [164]. Even then, such reagents are not stable for much longer than an hour.

The PD treatment process is a lengthy one that consists of numerous immersion baths and several reagents [168]. Up to two hours may be required to complete the whole procedure. Due to the number of aqueous immersion baths involved in this method, PD is implemented only after documents have been treated with amino acid-sensitive reagents, such as ninhydrin, in order to avoid washing away the amino acids before fingermark detection [159, 164]. PD is also employed at the end of a detection sequence due to the destructive and irreversible treatment mechanisms involved [159].

1.4.2 Oil Red O

Oil red O (ORO; 1-([4-(xylylazo)xylyl]azo)-2-naphthol; Figure 1.8) is a lipophilic dye that is structurally related to the Sudan group, a class of dyes employed in histological staining techniques. It was first manufactured for use as an industrial dye, employed in timber staining [169]. The utilisation of ORO as a histological stain was first reported in the late 1920s by French [169, 170]. ORO is used to stain tissue sections, such as adipose tissue, to demonstrate lipid content [167, 171]. Initial solvents for histological preparations of ORO included acetone and ethanol. These have largely been replaced by isopropanol and propylene glycol, which dissolve out less lipid material from the cells [172].

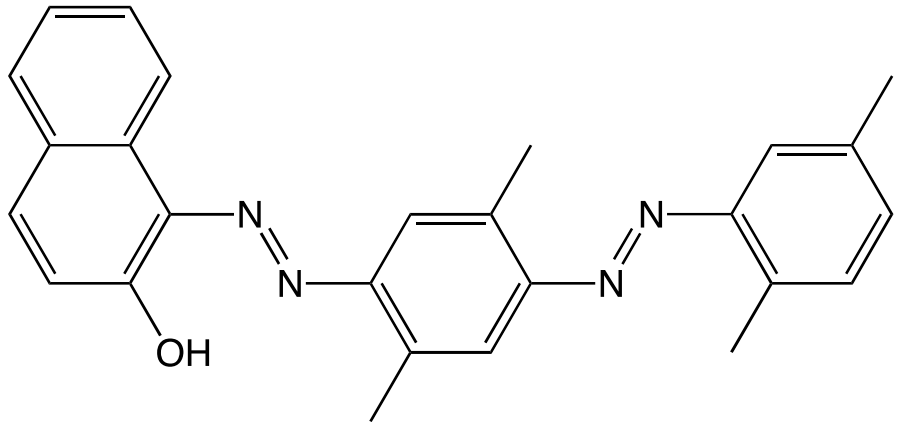

Figure 1.8: Molecular structure of Oil red O

ORO is a relatively new reagent for latent fingermark development. Its first application to forensic evidence was as a means of detecting latent lip prints by dusting powdered dyes over a surface, similar to dusting for latent fingermarks [173, 174]. In 2004, Beaudoin reported the first adaptation of ORO as a fingermark development reagent, as a possible alternative to PD [171]. As PD is currently one of the only conventional fingermark

development techniques that can detect fingermarks on porous surfaces that have been exposed to water, a simpler, effective alternative would be desirable. The ORO method proposed by Beaudoin has several advantages over PD in that it is more stable, easier and less time-consuming to use, and produces superior results on some substrates, on both dry and wetted porous surfaces [159, 175, 176].

Beaudoin's ORO formulation is based on that used to stain lipoproteins following cellulose acetate electrophoresis [159]. Fingermark development involves immersing the substrate in the staining solution for 60 – 90 minutes, producing red-stained fingermarks on a light pink background [167]. This is followed by rinsing the sample, first in a pH 7 buffer to neutralise the alkaline ORO reagent, in order to stabilise and preserve the developed fingermarks, followed by rinsing twice in deionised water [159]. The carbonate buffer originally proposed as a neutralisation reagent has recently been replaced with a more stable phosphate buffer [177].

The development mechanism is the simple diffusion of ORO from the solvent into the lipid fraction of latent fingermark residue [159, 177]. ORO is therefore an effective fingermark development reagent as long as the fingermark contains a sufficient amount of sebaceous material. Fingermarks containing a small amount of lipid material may still be visualised by ORO, although the resulting fingermark development is often too faint to enable a comparison. Depending on the substrate type, the extent of background colouration may vary, but contrast between the fingermark and the substrate is usually sufficient. Results obtained by Salama *et al.* suggest that ORO may not be entirely lipid specific, though it remains to be seen whether or not this is due to the ORO itself, or whether the high concentration of sodium hydroxide in the reagent chemically alters the dye molecule [159]. Additionally, this high concentration of sodium hydroxide appears to be necessary to improve fingermark contrast [159].

ORO produces best fingermark development on white or light-coloured paper substrates. Treatment of thermal paper receipts with ORO causes the printed text to fade, which is advantageous in revealing fingermark detail, though precautions must be made to photograph the text first [175]. Patterned and dark-coloured substrates present the greatest challenge to ORO treatment, as the red fingermark ridges are often obscured on these surface types. ORO cannot develop fingermarks on adhesive substrates, as interactions between ORO and the glue result in a deep red stain that obscures any fingermark detail [159]. Very porous paper types such as newspaper, phone directory pages

and brown paper are also problematic for ORO development, as the fingermark often appears as an indistinct blotch [159, 175]. It has been suggested that substrate porosity may affect ORO fingermark development, as lipids may diffuse more rapidly through rough, porous papers, thereby obscuring the ridge pattern of the fingermark [175]. This is consistent with the observation that fingermarks several weeks old detected with ORO on white copy paper also appear as blotches [159].

Comparisons between ORO and PD have found that while ORO produces superior results to PD on fingermarks less than four weeks old, PD is the superior method for detecting older fingermarks [159]. ORO performed with decreasing efficacy when treating older fingermarks compared to PD. While PD can be used to detect latent fingermarks that are up to several decades old [162], ORO does not produce satisfactory ridge development on fingermarks that are more than a few weeks old [159].

1.4.3 Nile red

One of the greatest shortcomings in the detection of latent fingermark lipids on porous substrates is the lack of photoluminescent methods [178]. Neither PD nor ORO are effective at developing fingermarks on dark or patterned substrates where the colour of the fingermark does not provide sufficient contrast.

Nile red (9-diethylamino-5H-benzo[α]-phenoxazine-5-one; Figure 1.9) is a neutral phenoxazone dye that is used as a fluorescent probe for the staining of neutral lipids [179-181]. Its photoluminescent properties provide increased sensitivity and superior visualisation of tissue structures compared to non-photoluminescent lipid stains such as ORO [179, 180]. Nile red exhibits solvatochromism; i.e. its absorption and emission maxima vary with solvents of different polarities [181-183]. It is highly photoluminescent in non-polar media and to a lesser extent in some polar solvents, but photoluminescence is completely quenched in aqueous solution [180, 183]. This is thought to be due to a twisted intramolecular charge transfer process undergone by the diethylamino group [182, 183].

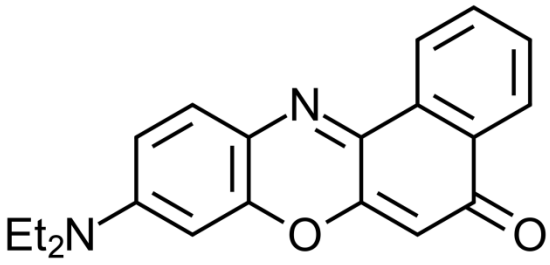

Figure 1.9: Molecular structure of Nile red

Nile red has been applied as a novel reagent in forensic applications, including as an enhancement technique for cyanoacrylate-fumed fingermarks, and as a potential reagent for the detection of latent lip imprints [184, 185]. Nile red was first reported as a latent fingermark development reagent by Saunders in 1993 [186]. A stock solution of Nile red in acetone was used to prepare an aqueous working solution. The resultant working solution was noted to have a very short shelf life of less than an hour [186].

More recent developments by Braasch *et al*. have produced an improved Nile red reagent that imparts both colour and photoluminescence to lipid-rich fingermarks, which appear as red on a non-luminescent purple background [187]. While this method has been reported to be an effective method of detecting recently deposited fingermarks on porous substrates that have been wetted, the authors noted concerns regarding the toxicity of the basic, methanolic solvent required due to the poor solubility of Nile red in water, which poses a problem to both personnel exposure and waste disposal [188]. Furthermore, the reagent suffers from significant precipitation of Nile red as the methanol portion of the solvent evaporates. A number of Nile red derivatives have been synthesised to overcome these issues, with the aim of producing a photoluminescent, water-soluble, lipophilic reagent [188].

1.5 Aims and overview

The fundamental aim of this thesis is to investigate the lipid fraction of latent fingermarks, in regards to the use of lipophilic dyes as novel development reagents, as well as compositional variation with individual traits and degradation, and how this variation may affect their detection. Obtaining more thorough knowledge of the chemical composition of latent fingermarks is an important step towards understanding the chemical and physical

mechanisms of the various fingermark development techniques. There is also potential to establish methods of obtaining additional information from fingermark composition such as donor age, or the age of a fingermark itself.

Chapter 3 describes the development of novel reagents for latent fingermark development on porous substrates, based on two lipid-sensitive histological stains, Oil red O and Nile blue. Additionally, this chapter outlines the evaluation of the potential applications of each of these methods, in comparison to established and novel reported techniques. Further investigations in Chapter 4 focus on the performance of Oil red O compared to physical developer, using fingermark samples collected from a large donor population. These results will be used to determine any correlations between fingermark development and donor traits, as a function of latent fingermark composition.

Chapter 5 examines variation in fingermark lipid composition in an effort to establish whether correlations exist between lipid profiles and donor traits, using a statistically relevant sample population. To this end, an analytical method was developed using gas chromatography-mass spectrometry in conjunction with chemometric methods. Chapter 6 describes the application of the same method to explore compositional variations related to fingermark age. Furthermore, Chapters 4 and 5 discuss the difficulties associated with designing large-scale fingermark investigations for both validation of development reagents and analytical studies.

Chapter 2: Experimental considerations

Portions of this chapter have been published in *Journal of Forensic Identification:*

Frick, A.A., P. Fritz, S.W. Lewis, W. van Bronswijk , *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.

Frick, A.A., P. Fritz, S.W. Lewis, W. van Bronswijk, *Sequencing of a modified Oil Red O development technique for the detection of latent fingermarks on paper surfaces.* Journal of Forensic Identification, 2013. **63**(4): p. 369-385.

Zadnik, S., W. Van Bronswijk, A. A. Frick, P. Fritz, and S. W. Lewis. *Fingermark simulants and their inherent problems: A comparison with latent fingermark deposits*. Journal of Forensic Identification, 2013. **63**(5): p. 593-608.

2.1 Introduction

Paper-based items that are examined for latent fingermarks may be collected after having been exposed to water or high humidity, which curtails the efficacy of the most commonly-used methods of fingermark development on these substrates; the amino acid-sensitive reagents [30, 159, 189]. Currently, the only development method in routine use for wetted porous substrates is physical developer (PD), which presents a number of issues in its cost and instability [159, 171, 190]. Consequently, there is a need to develop alternative methods that target the water-insoluble fraction of latent fingermarks. Several such reagents have been proposed, most notably the lipophilic dyes Oil red O (ORO) and Nile red [171, 187]. However, the development of a novel fingermark detection method is rather more complicated than it might initially seem. Approaches in fingermark research can vary significantly between studies, with little agreement in regards to experimental design, sample collection and assessment protocols for the evaluation and comparison of established and novel methods [27, 191, 192]. This lack of standardised methodology can have significant effects on the outcome of development and validation studies. Detailed guidelines by the International Fingerprint Research Group (IFRG), released contemporaneously with the final stages of this thesis, highlight the necessity of a more uniform experimental approach [191]. Additionally, the collection of potentially sensitive identifying information from fingermark donors necessitates further considerations to protect donors' rights and privacy.

This chapter outlines the experimental considerations taken into account for the studies detailed in Chapters 3 and 4, as well as the experimental procedures utilised. The ethical considerations described below also apply to Chapters 5 and 6.

2.2 Latent fingermark collection

2.2.1 Ethical considerations

Approval of the sampling protocol by the Curtin University Human Research Ethics Committee (approval numbers SMEC-86-11 and SMEC-07-13) was granted prior to sample collection in the studies outlined in Chapters 3 – 6. Obtaining such approval is integral in ensuring protection of donors' privacy, due to the identifying nature of fingermarks and the additional information collected, such as age and biological sex [11, 191], as well as the donors' right to withdraw from the study if so desired.

In accordance with the approved protocol, potential donors were informed verbally and in writing of the aims of the research, as well as their rights regarding confidentiality and their right to withdraw consent at any stage of the project. Donors were provided with contact details of the researcher(s) involved in the project should they wish to ask any questions or request to withdraw from the project and have their samples, data and associated documentation destroyed. Provided that donors agreed to participate in the study, all donors were required to sign a consent form before any sample collection was carried out. In the case of donors under 18 years of age, parental or guardian consent was required instead. Examples of the information sheet and consent forms are provided in Appendix 1.

Fingermark samples were made anonymous by assigning an alphanumeric code to each donor. This code was the only information used to label samples. Consent forms, code assignation and donor information were stored separately from fingermark samples, and were only accessible by the researchers directly involved in these studies.

2.2.2 Sample collection

The lack of standardisation in sample collection and experimental design, in addition to the natural variability of latent fingermark deposits, can hinder meaningful comparisons of results between studies of latent fingermark development methods [27]. As a result, there have been calls in recent years for more rigorous standards in regards to the number of fingermark donors required, sample collection and treatment, to minimise experimental variation where possible [109, 191, 192].

Ideally, the number of donors and the number of fingermarks collected should be sufficient to derive statistically valid data, but practical constraints often restrict the number of donors to a handful of individuals, usually those working in the immediate vicinity of the researchers [27, 192]. This can have a bottleneck affect, where the sample population is not representative of the general population in terms of the quality of the fingermarks obtained [109, 192]. Guidelines recently proposed by the IFRG divide experimental approaches into four phases [191]. Phase 1, which encompasses basic, proof-of-concept studies, requires a minimum of 3 – 5 donors, who provide a range of fingermarks of good to poor development quality.

Fingermark research is complicated further by the inherent difficulties in obtaining reproducible samples [11, 27]. Samples collected from the same person at the same time may show significant variation in composition [14]. Latent fingermarks are difficult to deposit in a reproducible and homogenous manner, due to the uneven distribution of

eccrine and sebaceous components on the ridge skin [193]. Sample deposition may range from asking donors to touch the substrate briefly [11], to more controlled procedures that regulate the length of contact between the fingertip and the substrate, as well as the amount of pressure used in depositing the fingermark [12, 194, 195]. It is unclear what basis exists for the precise conditions used (aside from control over these variables or to produce a clear fingermark pattern), and as a result it is unclear whether this is representative of 'real' fingermark deposition.

A common approach in studies focused on the lipid fraction of latent fingermarks is to have donors rub the tips of their fingers on areas of skin that are dense in sebaceous glands, namely the forehead and nose, prior to fingermark deposition [10, 14, 159, 166]. Such actions are referred to as 'charging' of the fingertips, and are intended to deliberately incorporate lipid material into the deposited fingermarks [192]. This results in a significantly greater amount of material to be deposited, as well as over-representing the lipid fraction of fingermark residue, which may lead to incorrect conclusions regarding the performance of lipid-sensitive development methods [15, 27, 109, 192]. It has been suggested that charged fingermarks are not realistic of latent fingermarks encountered in forensic investigations, and that uncharged fingermarks should be used instead, or as a comparison [15, 27, 191]. Frequently, cosmetics and other such products present on the skin surface will also be transferred to fingermark samples [11]. Another procedure is to clean donors' hands of any exogenous contamination before allowing time for skin secretions to replenish [193]. Acetone or alcohols are recommended as cleaning agents, as soaps may leave fatty acid residues on the skin [10, 109, 193].

For comparisons between development methods, a generally accepted approach is the 'split fingermark' (Figure 2.1), whereby fingermarks are cut in half, and each half is treated separately to enable a comparison [191, 192]. Another approach is the 'depletion series' method, where the donor is asked to deposit several fingermarks sequentially, without re-charging or allowing eccrine secretions to re-accumulate on the fingertips. In this way, a sequence of fingermarks containing diminishing amounts of material is obtained, which enables assessment of the sensitivity of a development method [27, 192].

Figure 2.1: Example of a split fingermark on white copy paper, treated with PD (left) and ORO (right)

2.2.3 Storage

Many studies into development reagents use relatively 'fresh' latent fingermarks, which are treated within a short period of collection (usually hours). In an operational context, items may not be examined for latent fingermarks until several days to weeks after deposition [191, 192]. Humidity, temperature and light can affect fingermark composition over prolonged storage periods [109, 192]. If samples are to be stored in the laboratory, there is a risk that some fingermark development may occur during storage if samples are kept in close proximity to reagents such as 1,2-indanedione. These factors must therefore be taken into account if fingermarks are intended to be developed after a prolonged period of time.

2.3 Reagent formulation

Continued investigations of fingermark development reagents aim to improve not only current fingermark detection capabilities, but also factors such as simplicity of the method, cost-effectiveness and operational safety, which must be considered in the context of routine, operational use. Both the ORO and Nile red development reagents were developed as simpler, less expensive alternatives to PD, but utilise alkaline methanol as a solvent [171, 187]. Considering that operational use of these reagents would be performed by non-

scientifically trained personnel, the toxicity of the organic solvent required may pose a concern. Less hazardous alternatives would therefore be desirable. Additionally, Nile red is a rather expensive reagent (>A$600/g), which may limit its use as an operational method [188].

In the case of PD, minor modifications to the working solution have been necessitated by the availability of some components. Synperonic N, a non-ionic surfactant, is added to the redox solution to facilitate the solubility of n-dodecylamine, which is in turn required to stabilise the redox system [29, 196]. Synperonic N, once a widely used industrial surfactant, is no longer manufactured in Europe due to concerns regarding its persistence in the environment and biodegradation to oestrogenic compounds [166, 197, 198]. The banning of Synperonic N use in many countries has prompted investigations into alternative non-ionic surfactants to replace Synperonic N in PD. Work carried out by the United States Secret Service (USSS) indicates that Tween 20 is an effective substitute, and that the USSS working solution is stable for a significantly longer period of time than UK version [166, 199]. Similar results have been obtained at Curtin University [200]. It has been suggested that the non-ionic surfactant participates in the formation of the surfactant layer surrounding silver particles, and that due to its more complicated molecular structure, Tween 20 may be more effective in this role than Synperonic N [166].

2.4 Reagent quality control

Routine testing of fingermark development reagents is required to identify performance issues from degradation of aged reagents, improper preparation or contamination [201, 202]. A notable shortcoming in such procedures is the lack of analytical standards for quality control testing [109, 202, 203]. A common practice is to test reagents on latent fingermarks gathered from immediately available donors, however this leaves the assessment of a reagent's efficacy prone to error, due to the natural variability of skin secretions. Latent fingermarks are known to vary significantly in composition between individuals, and compositional differences have been observed from the same individual over time. This so-called 'donor effect' also prevents truly meaningful comparisons between reagent performances carried out in separate facilities into the effects of laboratory protocol, climate and substrate [109, 204].

While testing on latent fingermarks is still the preferred method for determining reagent performance, there have been several preliminary attempts towards producing a

reproducible artificial fingermark that may be used as a uniform standard [25, 192]. Nielson proposed three criteria for such 'standard fingermarks': they must allow quantitative and qualitative testing; must reasonably reflect fingermark composition; and must be easily reproducible [204]. The current state of standard development is the use of spot tests or test strips – standard solutions of a target compound deposited onto paper by either micropipette or inkjet printer, and then treated with the relevant development method. Such tests have been developed largely for amino acid-sensitive reagents, although similar tests for PD have been reported [190, 192, 202, 203, 205-207]. These tests are limited to only a handful of reagents at best, and due to their simple composition do not accurately reflect latent fingermark composition, or the performance of the tested reagents on actual fingermarks [27, 109, 203, 208]. These tests can only be considered to reliably indicate the efficacy of the development reagent(s) in responding to the test itself. In the case of PD, spot tests have been developed using ethylenediaminetetraacetate (EDTA), which reacts rapidly with the working solution but is not present in latent fingermarks [202, 208]. The use of EDTA rather than a more realistic standard is due in part to a lack of understanding of the target compounds of this reagent.

2.5 Visual recording and assessment of developed fingermarks

Developed fingermarks must be recorded photographically. If fingermarks are treated with several development methods in a detection sequence, photographing any development following the application of each method is a necessary step to maintain a record of ridge detail, in the event that further treatment impairs any fingermark development produced by a preceding technique. Additionally, some development reagents are not stable post-treatment, and deterioration of ridge detail quality may begin to occur within hours in some cases [187].

The quality of developed fingermark ridge detail is usually assessed visually. There are several fingermark grading schemes currently in use by researchers and industry professionals alike, tailored to suit specific purposes, such as comparisons between two development methods, or overall assessment of the quality fingermark development [27, 35, 176, 192, 209-212]. Generally, such assessment methods consist of the categorisation of developed fingermarks along a scale ranging from 'good' to 'poor' ridge detail. For example, a grading scale proposed by McLaren *et al.* is used to compare the performance of one development reagent against another, along a range of -2 (major decreased quality compared to control) to +2 (major improvement compared to control) [210]. Another

commonly employed system is that devised by the Home Office Police Scientific Development Branch (HOPSDB), UK [211]. This system assigns absolute values to fingermark development, using a scale of 0 (no development) to 4 (full development with continuous ridges and excellent contrast). A third scale reported by Becue *et al.* is designed to evaluate the usefulness of a developed fingermark to identification, assigning fingermarks a grade of – (no ridge development), ± (some visible development) and + (sufficient development to enable identification) [212].

2.6 Experimental

This section outlines the general fingermark development procedures followed throughout the course of this thesis. Additional procedures and method development are described in the relevant chapters.

2.6.1 Chemicals and reagents

Oil Red O (dye content ≥75 %; Sigma-Aldrich, USA), propylene glycol (≥99 %; Sigma-Aldrich, USA), maleic acid (≥99 %; Sigma-Aldrich, USA), silver nitrate (≥99.5 %; Chem-Supply, Australia), ferric nitrate nonahydrate (≥98 %; Sigma-Aldrich, USA), ferrous ammonium sulphate hexahydrate (99 %; Sigma-Aldrich, USA), citric acid (≥99 %; Ajax Finechem, Australia), Tween 20 (≥40 %; Sigma-Aldrich, USA) and n-dodecylamine acetate (Optimum Technology, Australia) were all used as received and were of analytical reagent grade unless otherwise stated.

2.6.2 Preparation of reagent solutions

2.6.2.1 Oil red O

The preparation of ORO was adapted from the method by Chiffelle and Putt [213]. 0.05 g ORO was dissolved in 100 mL propylene glycol with constant stirring at 95 °C. The solution was left to cool slightly before undissolved ORO was removed using vacuum filtration, and left to stand until completely cooled before use. The ORO solution was stored at room temperature in Schott bottles wrapped in aluminium foil.

2.6.2.2 Physical developer

Physical developer stock solutions and working solution were prepared following the UK-PD formulation as described by the Australian Federal Police (AFP) [168] with the following modification: Tween 20 was substituted for Synperonic N, as described in Sauzier *et al*. [200]. Preparation of all physical developer reagents is outlined in Table 2.1.

Table 2.1: Composition of PD stock solutions and working solution [168]

Solution	Reagent preparation
Detergent-surfactant solution	2 g n-dodecylamine acetate and 2 g Synperonic N dissolved in 500 mL deionised water
Redox solution	30 g ferric nitrate nonahydrate, 80 g ferrous ammonium sulphate hexahydrate, 20 g citric acid and 40 mL detergent-surfactant solution dissolved in 900 mL deionised water in the order given
Silver nitrate solution	10 g silver nitrate dissolved in 50 mL deionised water
Maleic acid pre-wash	25 g maleic acid dissolved in 1 L deionised water
Working solution	12 mL silver nitrate stock solution added to 237 mL redox stock solution

The silver nitrate stock solution was stored in a Schott bottle wrapped in aluminium foil. The working solution was prepared fresh for each use, and used twice before discarding.

2.6.3 Reagent quality control tests

2.6.3.1 Oil red O

1 % and 0.1 % v/v solutions of linseed oil were prepared in hexane. 10 µL of each solution was pipetted onto a strip of white copy paper and left to air dry. The test strips were immersed in ORO solution for 15 minutes. After 15 minutes, the test strips were removed, rinsed twice with deionised water and left to air dry. 1 % and 0.1 % linseed oil solutions should appear as dark and light pink spots, respectively (Figure 2.2).

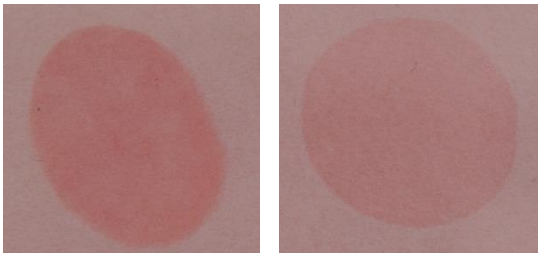

Figure 2.2: 10 µL aliquots of 1 % (left) and 0.1 % (right) linseed oil on white copy paper, treated with ORO

2.6.3.2 Physical developer

Preparation of the physical developer reagent test was based on the method described by Houlgrave *et al*. [202]. 3 g EDTA disodium salt was dissolved in 90 mL deionised water. Sodium hydroxide pellets were added until EDTA had completely dissolved (5 – 7 pellets). A tenfold dilution of the EDTA solution was then prepared. 10 µL of each EDTA solution was pipetted onto filter paper and left to dry. Once dry, test papers were immersed in the physical developer working solution until the reaction was complete (approximately 3 seconds). Undiluted EDTA should be black/dark grey in appearance, while diluted EDTA should be a light grey colour (Figure 2.3).

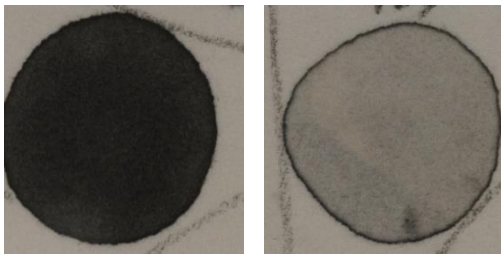

Figure 2.3: 10 µL aliquots of undiluted (left) and diluted (right) EDTA on filter paper, treated with PD

2.6.4. Sample preparation and collection

Latent fingermark collection was carried out using the following procedure unless otherwise stated. For charged fingermarks, donors were asked to briefly rub their fingertips across their forehead and/or the bridge of their nose. For uncharged fingermarks, donors were asked to simply deposit fingermarks onto the substrate. Fingermarks were deposited by gently pressing the fingertips to the substrate for approximately 5 seconds. Donors had not consumed food or handled chemicals for at least 30 minutes before providing samples. White copy paper (Fuji Xerox Professional) was used as the substrate. Fingermark samples were treated within 36 hours following deposition. Unless otherwise stated, samples were collected from the minimum number of 3 – 5 donors specified by the IFRG guidelines.

Samples not treated immediately following deposition were placed in separate paper envelopes and stored in a closed laboratory cupboard away from reagent containers, developed fingermarks, or any other possible sources of contamination. No attempts were made to control storage conditions.

2.6.5 Development of latent fingermarks using Oil red O

Samples were placed in a glass tray and immersed in ORO reagent for 15 minutes, with manual agitation provided by gently rocking the tray for 30 seconds at the beginning of treatment. After development, ORO treated samples were rinsed twice in a deionised water bath under running water, and air-dried on paper towels at room temperature.

The development of this optimised method was the result of investigations outlined in Chapter 3.

2.6.6 Development of latent fingermarks using physical developer

Treatment with PD was carried out as described by the AFP with one minor modification [168]. During familiarisation with the technique, it was found that silver deposited rapidly onto the paper substrate as grey-black patches, obscuring fingermark development in some places. The immersion of the samples in maleic acid as stated in the AFP manual was determined to be an insufficient length of time for complete removal of carbonate fillers in the substrate. The maleic acid pre-treatment step was increased from 5 minutes to 30 minutes (until the formation of bubbles from the substrate ceased), as recommended by Salama *et al.* [159]. Each step of the development process was carried out in a separate glass tray. Fingermark samples were treated by first being rinsed twice in deionised water for 10 minutes, then immersed in maleic acid as described above, and rinsed again in deionised water for 10 minutes. Samples were then immersed in the working solution, and removed once satisfactory fingermark development was observed or after 30 minutes. After development, PD-treated samples were rinsed several times in deionised water and air-dried on paper towels at room temperature, away from direct light.

2.6.7 Visual recording and assessment of developed latent fingermarks

Samples were photographed using a Nikon D300 camera, equipped with an AF-S Micro-Nikkor lens, mounted on a Firenze Mini Repro tripod and connected to a computer using Nikon Camera Control Pro Version 2.0.0. ORO and PD treated samples were photographed in reflectance mode (Table 2.2). Illumination was achieved using incandescent light bulbs with no camera filter attachments.

Table 2.2: Photographic conditions for fingermarks treated with Oil red O and physical developer

Focal length (mm)	Exposure mode	White balance	Shutter speed (s)	Aperture	Sensitivity
60	Manual	Auto	1/20	f/11	ISO 200

Treated fingermarks were graded using a system based on that used by the Home Office Police Scientific Development Branch (HOPSDB), UK (Table 2.3) [211]. Later adjustments of the images for contrast and brightness were performed using Adobe Photoshop CS4 Version 9.0.

Table 2.3: Grading system for developed latent fingermarks

Grade		Description
0	No development	No visible ridge detail
1	Weak development	Signs of contact, but less than 1/3 of fingermark visible as continuous ridges
2	Medium development	1/3 – 2/3 of fingermark visible as continuous ridges
3	Strong development	More than 2/3 of fingermark visible as continuous ridges, but not quite a 'perfect' fingermark
4	Full development	Whole fingermark visible as continuous ridges

Chapter 3: Lipid-sensitive development reagents derived from histological stains

Portions of this chapter have been published in *Journal of Forensic Identification* and *Chemical Communications*:

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Frick, A.A., P. Fritz, S.W. Lewis, W. van Bronswijk, *Sequencing of a modified Oil Red O development technique for the detection of latent fingermarks on paper surfaces.* Journal of Forensic Identification, 2013. **63**(4): p. 369-385.

Frick, A.A., F. Busetti, A. Cross, S.W. Lewis, *Aqueous Nile blue: a simple, versatile and safe reagent for the detection of latent fingermarks.* Chemical Communications, 2014. **50**(25): p. 3341-3343.

3.1 Introduction

As discussed in Chapter 1, there are a limited number of development methods in operational use that interact with the water-insoluble fraction of latent fingermarks on porous substrates. Physical developer (PD) is currently the method of choice, but despite its effectiveness, the PD working solution remains notorious as an unstable and difficult reagent to work with [121, 171, 176, 190]. This, together with the time-consuming nature of the treatment process, limits PD from being in widespread, routine use unless absolutely required [165].

There has been some work in recent years focused on the adaptation of histological stains to latent fingermark detection. Dyes used in the demonstration of lipids provide simple and relatively inexpensive fingermark development reagents. Sudan black and gentian violet have been utilised in the detection of fingermarks on porous and non-porous surfaces, and adhesive surfaces, respectively [186, 214, 215]. More recently, Oil red O (ORO) and Nile red have been proposed as development reagents for porous substrates, as alternatives to PD [171, 187]. These methods are generally less time-consuming, less hazardous, are more cost-effective, and provide comparable results to PD with recently deposited latent fingermarks. Additionally, Nile red is a photoluminescent reagent, which provides a significant advantage over PD and ORO in terms of sensitivity and applicability to a wider range of substrates.

3.1.1 Oil red O

Beaudoin's ORO method, described in Chapter 1, is easier to use than PD, but still remains time-consuming, complex, and less sensitive when compared to amino acid-sensitive reagents [171, 176]. Additionally, a large amount of undissolved ORO must be filtered out before the solution can be used, indicating that the reagent formulation may require further optimisation. Alcohol solutions can dissolve out some of the lipids present in cells, and may have the same effect on latent fingermarks, which has prompted some investigations into reformulating the reagent [159]. As latent fingermarks are composed of trace amounts of eccrine and sebaceous residues, their potential dissolution may greatly affect fingermark detection.

Commonly used solvents for ORO histological stains include isopropanol [216-218], propylene glycol [213, 219-222], ethanol [223, 224] and triethyl phosphate [225, 226]. Salama *et al.* investigated whether the isopropanol and ethanol staining solutions might be viable alternatives to Beaudoin's formulation, and found that the two performed poorly in

comparison to the methanol solvent [159]. In histological applications, propylene glycol may yield better results than other commonly used solvents [172, 219]. This advantage may also be applicable to latent fingermark detection.

Initial comparisons between Beaudoin's ORO and a propylene glycol histological stain were carried out by Patrick Fritz at Curtin University as part of his honours dissertation [227]. Fingermark halves treated with the histological stain (0.5 g/100 mL ORO in propylene glycol) consistently showed superior contrast and sensitivity compared to those treated with Beaudoin's reagent for the same amount of time (Figure 3.1). These results indicated that propylene glycol might be a viable alternative solvent to Beaudoin's alkaline methanol solvent [227]. Additionally, the propylene glycol formulation requires fewer, less toxic components, potentially making it a more attractive option for operational use.

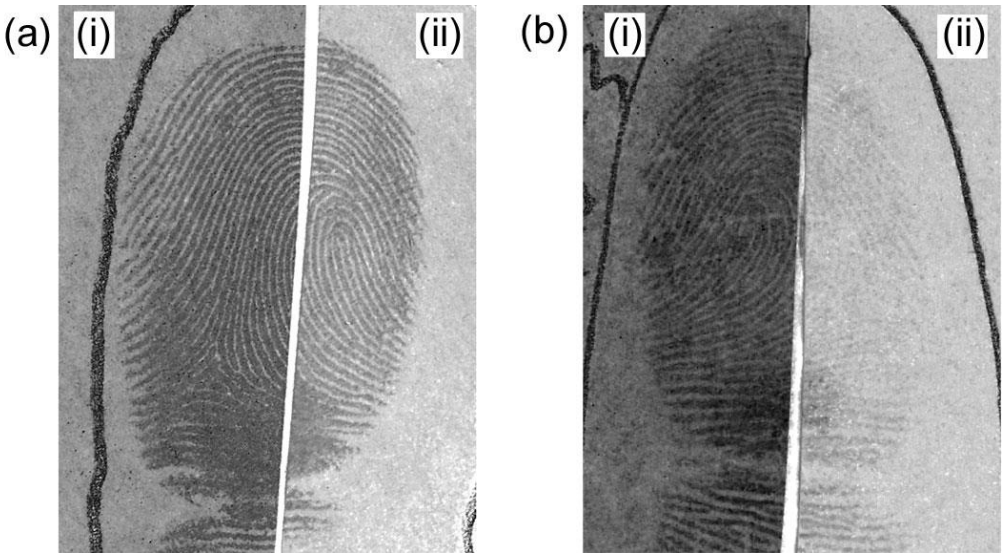


Figure 3.1: (a) Charged and (b) uncharged latent fingermark halves on white copy paper treated with (i) ORO in propylene glycol and (ii) Beaudoin's ORO for 15 minutes
(Figures provided courtesy of Patrick Fritz)

3.1.2 Nile blue A

As described in Chapter 1, while Braasch *et al.*'s Nile red reagent has been reported to be an effective method of detecting latent fingermarks on porous substrates that have been wetted, the authors raised concerns regarding the toxicity of the methanol solvent [188]. These concerns have prompted investigations into synthesising a number of Nile red derivatives, with the aim of producing a photoluminescent, water-soluble, lipophilic reagent [188].

Nile blue A (Nile blue sulfate; Figure 3.2), commonly referred to as Nile blue[1], is a basic phenoxazine dye employed primarily in histology to demonstrate acidic and neutral lipids [228]. Its first use as a tissue stain was reported by Smith *et al.*, who noted the ability of Nile blue A to distinguish between neutral and acidic lipid components when prepared as a 1 – 2 % aqueous solution [229, 230]. Nile blue stains acidic components, such as phospholipids and nucleic and fatty acids, a dark blue colour, while neutral lipids (i.e. triglycerides) are stained pink or red, and exhibit photoluminescence [213, 228, 231, 232].

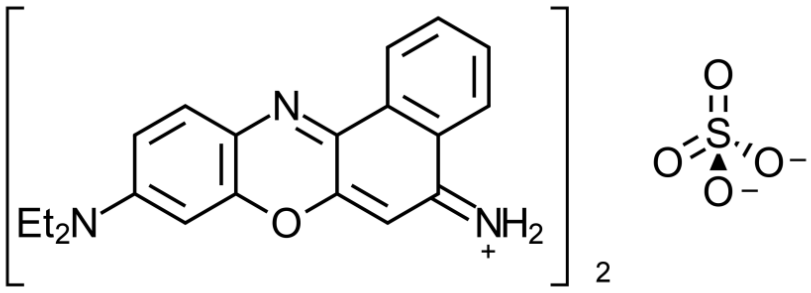


Figure 3.2: Molecular structure of Nile blue A

The dual staining capability of Nile blue is due to the spontaneous hydrolysis of Nile blue A in aqueous media to its corresponding phenoxazone, Nile red (Figure 3.3). The two dyes interact with their respective target compounds by different mechanisms: Nile blue A forms a salt linkage with acidic moieties, while Nile red dissolves preferentially into neutral lipids. Though Nile red is present in the Nile blue histological stain in only trace amounts, this is sufficient to provide colouration to stained tissue sections [179, 180]. It is accepted that the Nile red component is responsible for the photoluminescence emitted by tissues stained with Nile blue reagent [180, 233-235].

[1]The term 'Nile blue' is often used interchangeably to refer to both the dye Nile blue A, and the histological stain prepared from the same. For clarity, the term 'Nile blue' is used hereon specifically in reference to the aqueous solution, while 'Nile blue A' refers to the phenoxazine dye.

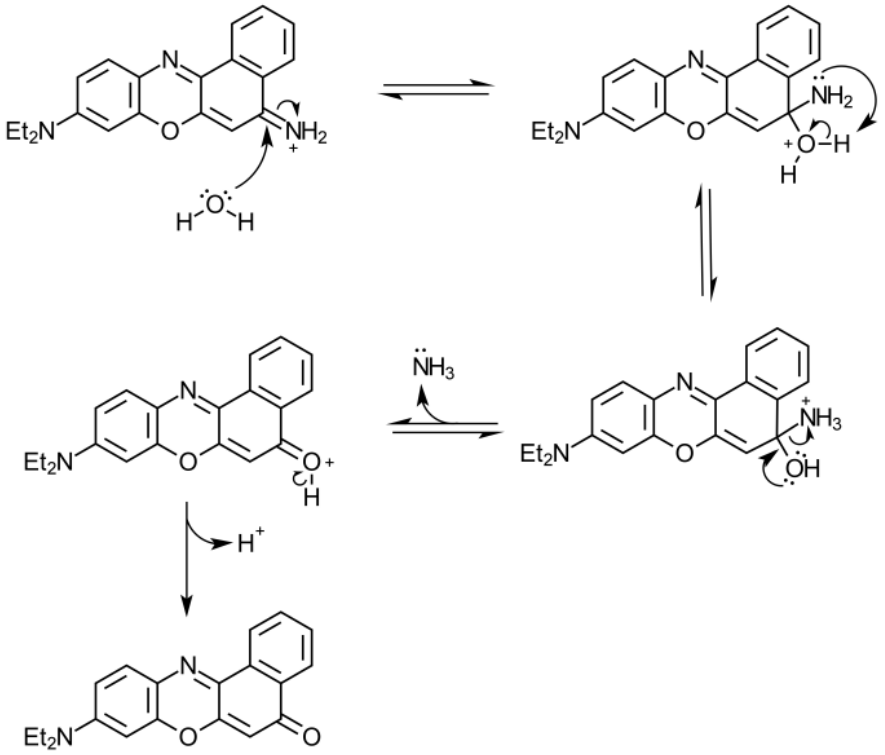


Figure 3.3: Hydrolysis of Nile blue A to Nile red in aqueous solution

Nile blue A in alcoholic solutions has been explored as a fingermark development reagent for use on non-porous substrates, including as a post-treatment stain for cyanoacrylate fuming of latent fingermarks [236], and for the detection of latent lip prints [185]. Nile blue A perchlorate has been previously applied to laser detection of latent fingermarks on non-porous and semi-porous substrates when prepared as a fluorescent, magnetic powder [237].

In the context of fingermark detection, the utilisation of an aqueous Nile blue A solution, rather than being a novel reagent in itself, may be viewed as a simple method of preparing an aqueous Nile red solution, and so negates the need to synthesise a water-soluble Nile red derivative. While Nile red is not water-soluble, it is soluble in trace amounts in aqueous Nile blue A [228]. Therefore, Nile blue A presented an attractive potential alternative to Nile red, and studies were conducted to investigate its potential to develop latent fingermarks on porous substrates. A more pragmatic advantage is that Nile blue A is significantly less expensive than Nile red. Prices given by Sigma-Aldrich as of December 2014 list 1 g of technical grade Nile red at A$654, while 25 g of Biological Stain Commission certified Nile blue is priced at A$139. As Nile Red is a rather expensive reagent, its cost may discourage its widespread use [178].

3.1.3 Aims

This chapter describes the development of two novel lipid-sensitive reagents, based on the histological stains ORO and Nile blue, for the detection of latent fingermarks. A propylene glycol-based ORO reagent, proposed by Patrick Fritz, was modified and applied to a range of porous substrates. The compatibility of this reagent with operational methods for fingermark detection on porous substrates was also examined. Preliminary investigations were conducted towards the first adaptation of Nile blue into a formulation suitable for latent fingermark detection on porous substrates.

3.2 Oil red O

3.2.1 Experimental

3.2.1.1 Sample collection

Unless otherwise stated, charged latent fingermarks were collected on white copy paper as described in section 2.6.4. Charged latent fingermarks were collected as described in section 2.6.4. For substrate investigations, samples were collected on a variety of porous substrates. A complete list of the paper types examined is given in Table 3.1.

Table 3.1: Porous substrates examined throughout ORO investigations

Substrate	Manufacturer/Product
White copy paper	Fuji Xerox Professional, Reflex A4 White
Coloured copy paper	Optix
Lined notepaper	Spirax Notebook
White envelope	Office National
Gold envelope	Unknown manufacturer
Post-it notes	Post-it
Thermal paper (unprinted and printed receipts; both sides of paper examined)	Officeworks, various unknown manufacturers
Newspaper	The West Australian
Phone directory	Yellow Pages Directory
Brown paper	Unknown paper bag manufacturer
Patterned wrapping paper	Unknown manufacturer

3.2.1.2 Chemicals and procedures

A number of materials and methods (Oil red O and physical developer) used in these investigations have previously been described in section 2.6.

For method development, a range of ORO concentrations (0.01 – 0.5 g/100 mL) were invesitgated. Divided (e.g. halved or quartered) fingermark samples on white copy paper were immersed in the reagent for up to 30 minutes, with manual agitation provided by gently swirling the samples in the ORO reagent for 30 seconds every 5 minutes, once at the beginning of development time, or no agitation.

The following reagents were used to prepare Beaudoin's ORO reagent: methanol (Mallinckrodt Chemicals, USA), sodium hydroxide (≥97 %; Ajax Finechem, Australia), concentrated nitric acid (70 %; Ajax Finechem, Australia) and sodium carbonate (≥99.9 %; Merck, Australia), which were all used as received and were of analytical reagent grade unless otherwise stated.

Beaudoin's ORO reagent was prepared following the procedure outlined by Salama *et al.* [159]. 1.54 g ORO was dissolved in 770 mL methanol and added to 9.2 g sodium hydroxide dissolved in 230 mL deionised water. To prepare the carbonate buffer, 26.5 g sodium carbonate was dissolved in 2 L of deionised water. 18.3 mL concentrated nitric acid was added to the sodium carbonate solution, and the buffer was made up to 2.5 L with deionised water.

Fingermark development with Beaudoin's ORO was carried out as described by Salama *et al*. [159]. Samples were placed in a glass dish and immersed in ORO for 60 minutes, then dipped briefly in the buffer solution. After development, samples were rinsed twice with running deionised water, and air-dried on paper towels at room temperature.

3.2.3 Results and discussion

3.2.3.1 Method development

Concentration

Supersaturated ORO solutions are commonly used as histological stains in order to render their targets brightly coloured and easily distinguishable from surrounding tissue. It was found that a large amount of ORO remained undissolved during preparation of these reagents and filtering this excess proved to be laborious and time-consuming. More ORO precipitated out of solution within a few days after preparation, creating a significant

detrimental impact on reagent performance. Lower concentrations were investigated to achieve a compromise between reagent stability and fingermark development.

Treatment of fingermarks with solutions containing more than 0.1 g/100 mL ORO produced intense colour and contrast within 15 minutes. These reagents also produced darker substrate staining than the lower concentration solutions. As the fingermark itself also appeared darker, this was not seen as a problem; however, none of these reagents were considered stable due to the large amounts of precipitate formed during storage. Solutions of 0.02 g/100 mL ORO or less failed to develop ridge detail to any satisfactory degree even after a 60 minute immersion period (grade 0 – 1), making these solutions impractical as fingermark development reagents. It was found that 0.05 g/100 mL ORO was the most efficient concentration, as this amount of dye dissolved almost completely in propylene glycol, and provided satisfactory staining intensity for most strong and weakly charged fingermarks examined. Additionally, far less precipitation occurred during storage than with the initial supersaturated formulation, making this concentration the most suitable for further studies.

Development time

Immersion for short periods of up to 10 minutes produced ridge development only on some fingermarks, presumably containing a significant amount of sebaceous material, otherwise sufficient ridge development was not achieved (average development grade of 0 – 1). The most effective contact time was found to be 15 minutes, which produced a greater number of higher quality fingermarks (grades 2 – 3). No discernible difference was observed between fingermark quarters treated for 15 – 30 minutes, with these contact times giving comparable contrast and colour intensity for even weakly charged fingermarks. Varying the development time did not appear to have any significant effect on the degree of substrate staining, which was more greatly influenced by reagent concentration.

Agitation

Constant agitation was originally recommended for treatment with Beaudoin's formulation, though it has since been found to be optional provided that the samples are completely immersed in the reagent. Constantly shaking the sample during development may result in overdeveloped fingermarks [159, 177]. Patrick Fritz's initial investigations into mechanical agitation obtained similar results using the propylene glycol reagent [227]. It was found that manual agitation for 30 seconds at the beginning of the development time produced

slightly darker fingermark ridges. Further manual agitation was not observed to significantly improve fingermark development quality (Figure 3.4).

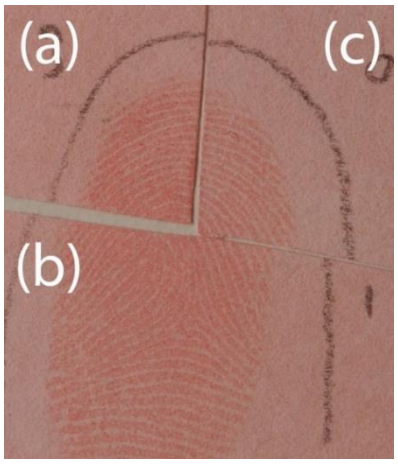


Figure 3.4: Latent fingermark thirds developed with ORO for 15 minutes, with (a) manual agitation every 5 minutes; (b) manual agitation at the beginning of development time; and (c) no agitation

Post-treatment

The clarity of stained tissue sections may be improved by employing a differentiation step to remove excess dye, i.e. rinsing the stained tissue in an appropriate solvent [172, 219, 220]. In an effort to improve the contrast between developed fingermarks and the substrate, an additional step of immersing ORO-treated fingermarks in propylene glycol was investigated. It was found that immersion in propylene glycol did remove some ORO from the substrate; however, fingermarks were also decoloured by this process, which not only failed to improve contrast, but reduced the clarity of the developed ridge detail by leaching ORO from the fingermark residue [224].

3.2.3.2 Development of samples exposed to water

Beaudoin's ORO has been demonstrated to successfully develop latent fingermarks on substrates which have been immersed for up to 1 week, as the reagent targets the hydrophobic lipid component of fingermarks [175, 176]. Work by Patrick Fritz indicated that while fingermark quarters immersed in deionised water maintained good contrast, clarity of fingermark ridge detail seemed to become washed away with increasing periods of submersion, often resulting in poorer quality fingermarks [227].

Subsequent investigations were carried out to establish whether or not ORO in propylene glycol could also develop fingermarks under these conditions. All wetted substrates treated with ORO produced a darker background than those treated dry, presumably due to the

paper fibres swelling during immersion, thereby increasing the porosity of the substrate and leading to greater absorption of ORO during treatment (Figure 3.5). This resulted in some loss of contrast with weakly charged fingermarks (grade 1).

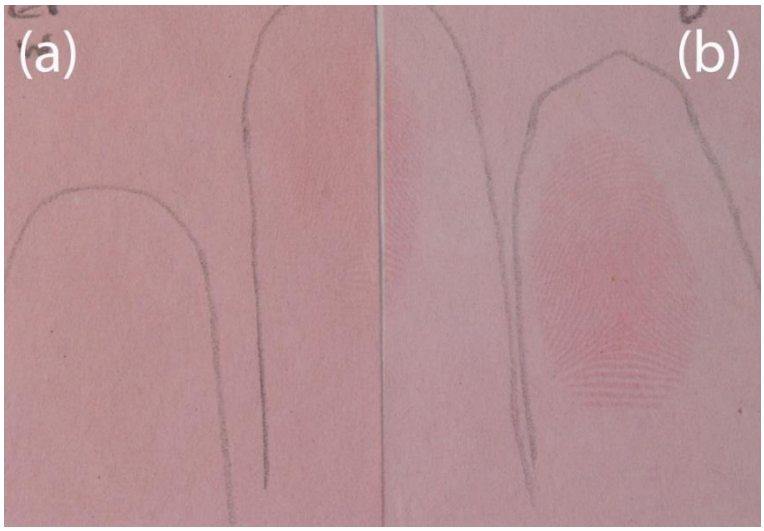


Figure 3.5: Fingermarks treated with ORO

(a) after 1 hour immersion in deionised water; and (b) dry

3.2.3.3 Substrate investigations

Fingermarks were deposited on a variety of porous substrates (Table 3.1) to investigate the versatility of ORO in propylene glycol as a fingermark development reagent. Fingermarks deposited on these substrates were cut in half and developed immediately or after being soaked in water for 1 hour as described above. While best fingermark development was achieved on white copy paper, fingermarks were successfully developed on most substrates examined, with results on different paper types similar to those obtained with Beaudoin's ORO [159, 175]. As with Beaudoin's ORO, ORO in propylene glycol was found to be unable to develop fingermarks on adhesive substrates such as sticky tape and Post-It notes, as interactions between ORO and the glue resulted in a uniform red stain that obscured any fingermark detail [159].

Variable results were achieved for fingermarks deposited on coloured substrates, such as coloured copy paper and Post-it notes, due to variations in the contrast between the treated fingermarks and the substrate. On dark-coloured or patterned substrates, although fingermark development was evident, the colour of the substrate prevented ridge detail from being clearly visible (grade 1 – 2). It must be taken into account that ORO stains the substrate as well as the fingermarks (e.g. yellow paper is stained orange), which can also affect contrast. This effect is more pronounced on wetted substrates.

ORO in propylene glycol was found to be effective on both sides of printed and unprinted thermal paper, though treatment of the active top layer of thermal paper resulted in uneven background colouration (Figure 3.6). Experiments showed that immersion in propylene glycol causes the print of thermal paper receipts to fade. Depending on the extent of the fading, which varied between receipts obtained from different sources, this may allow for better observation of ridge detail that would otherwise be obscured by text [175].

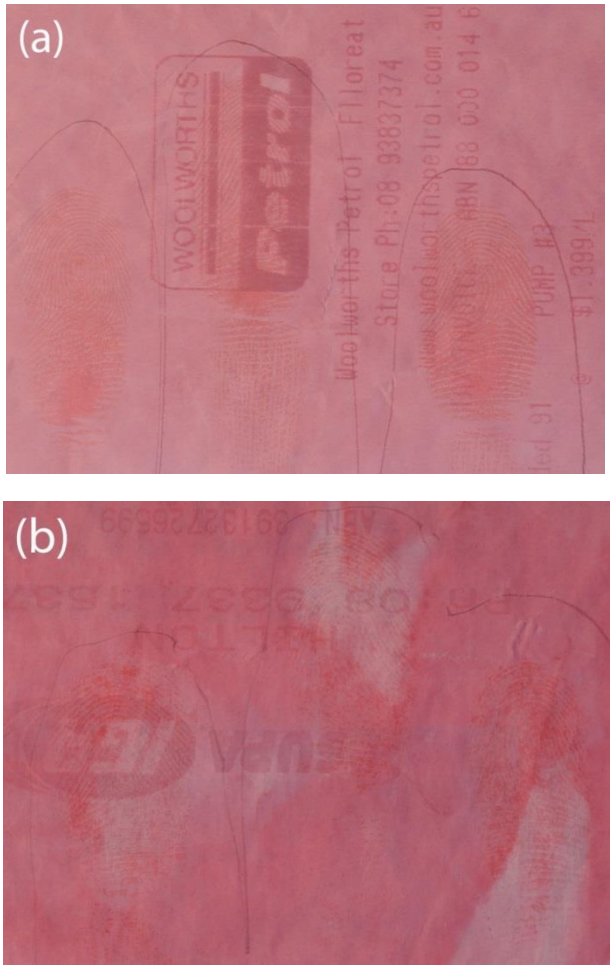


Figure 3.6: Fingermarks developed with ORO on thermal paper receipts showing a) fingermark development with some ridge detail visible though text; b) fingermark development partially obscured by uneven background discolouration

Newspaper, phone directory pages and brown paper bags proved to be problematic for fingermark development, as ridge detail was often obscured by the blotchy appearance of the fingermark (grade 1). Clear ridge detail could be obtained on some freshly-deposited fingermarks on these paper types, but not on those more than one day old. It has been suggested that substrate porosity affects ORO fingermark development, as lipids may

diffuse more rapidly through rough, porous papers, such as those described here, thereby obscuring the ridge pattern of the fingermark [175].

3.2.3.4 Comparisons to Beaudoin's ORO and physical developer

Charged fingermarks were halved and treated with ORO in propylene glycol and either Beaudoin's ORO or PD. For a direct comparison of all three development methods, charged and uncharged latent fingermarks from five donors were cut into thirds, and each piece treated with Beaudoin's ORO, ORO in propylene glycol, or PD.

While Beaudoin's ORO was found to produce greater colour intensity than ORO in propylene glycol on copy paper, both formulations produced a similar degree of contrast and ridge detail (Figure 3.7). The difference in colour intensity was not unexpected, as the two methods differ significantly in both length of immersion time and ORO concentration. A further possibility is that Beaudoin's ORO also may target other types of compounds found in latent fingermarks, in addition to neutral lipids. It is thought that the phenol group of ORO is deprotonated in the presence of sodium hydroxide, which may alter its staining properties in regards to selectivity [159]. The possible alteration of the ORO molecule is further evidenced by the colour of the development reagents: Beaudoin's ORO becomes burgundy in colour upon the addition of sodium hydroxide, whereas ORO prepared in propylene glycol remains a deep red.

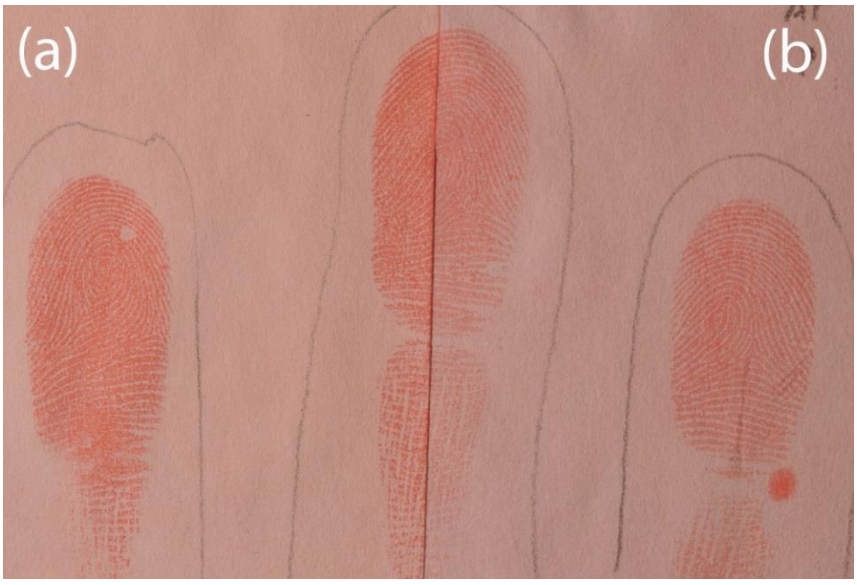


Figure 3.7: Fingermarks on white copy paper, treated with (a) Beaudoin's ORO for 60 minutes; and (b) ORO in propylene glycol for 15 minutes

Comparisons of the two ORO methods with PD showed that the performance of both ORO reagents was often equal to or better than that of PD on recently deposited, charged

fingermarks (Figure 3.8). However, on uncharged fingermarks, PD showed superior development compared to both ORO reagents, with some samples producing clear, though often patchy, ridge detail (grades 1 – 2) where both ORO reagents either failed to develop fingermarks or produced only very faint ridge detail (grades 0 – 1). Though PD is often described as a lipid-sensitive reagent, the deposition of silver particles is thought to be due to electrostatic attraction to water-resistant compounds including some proteins, as well as lipids, which may explain PD's ability to detect fingermarks with ostensibly minimal lipid content [164]. Beaudoin's ORO was again found to give slightly better results than ORO in propylene glycol on either type of fingermark, due to the more intense staining of fingermark lipids.

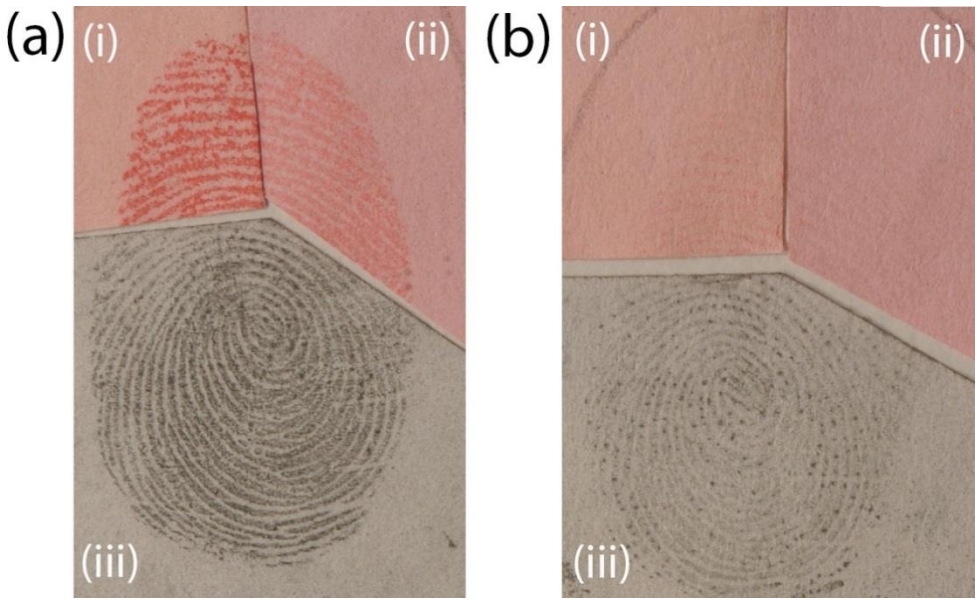


Figure 3.8: a) Charged and b) uncharged fingermark thirds treated with (i) Beaudoin's ORO; (ii) ORO in propylene glycol; and (iii) PD

Fingermarks stored for 2 months in the presence and absence of light were halved and treated with ORO in propylene glycol and PD. ORO performed poorly on all aged fingermarks, producing only a faint blotch of lipid material with no ridge detail (grade 0). ORO treatment was more effective on fingermark samples stored in the dark conditions than those stored in direct light. Twice as many fingermarks were detected, though all had the same blotchy appearance. While some samples could not be visualised with either method, PD treatment was found to outperform ORO on most samples, regardless of storage conditions, with superior results on samples stored in the dark (grades 1 – 2). The results achieved in this study are consistent with previous comparisons performed between PD and Beaudoin's ORO [159].

It has been hypothesised that the water-insoluble components present in latent fingermarks can be divided into two fractions, which goes some way to explaining the differences in performance between PD and ORO on older fingermarks [159]. PD is thought to react with the stable and long-lived 'robust fraction', composed of large, water-insoluble proteins and lipoproteins, which may interact with paper via hydrogen bonding, hence its ability to develop fingermarks that are several weeks (and up to several decades) old [159, 164, 166, 167]. The 'fragile fraction' is composed of more short-lived compounds such as fatty acids and triglycerides, which may be removed by some solvents of the amino acid-sensitive reagents, and is thought to be the target group of ORO [186]. Over time, the 'fragile' lipid fraction of latent fingermark residue diffuses through porous substrates, resulting in the blurred appearance of older fingermarks treated with ORO. Additionally, ORO permeates the entire substrate during treatment, staining all diffused lipids and thus creating a blotch of colour, while the accumulation of silver particles is thought to occur only at the surface of porous substrates, hence the ability of PD to detect fingermarks that are several months old in instances where ORO cannot [159].

3.2.3.5 Sequencing with 1,2-indanedione and physical developer

It is recognised that ORO cannot completely replace PD as a fingermark development method. The colour of the substrate, especially when text and patterns are present, affects the contrast of ORO-treated fingermarks to a greater extent than those developed with PD. PD also produces superior results to ORO on certain paper types, as well as on fingermarks which are over 30 days old. For these reasons, it has been suggested that ORO and PD be used in sequence, with ORO applied before PD, after the application of amino acid-sensitive reagents such as ninhydrin [159, 238]. Beaudoin's ORO has been successfully incorporated into development sequences with ninhydrin, DFO, 1,2-indanedione and PD [159, 238].

A series of investigations were carried out in collaboration with Patrick Fritz to evaluate the compatibility of ORO in propylene glycol in sequence with 1,2-indanedione and PD, and to determine the order in which ORO and PD should be applied. The order in which reagents are applied in a detection sequence should be such that the success of any reagent is not affected by the application of a preceding method [215]. As the aqueous immersion baths and rinses included in the ORO and PD methods would cause dissolution of amino acids present in latent fingermarks, 1,2-indanedione treatment was automatically placed first in the detection sequence.

Fingermark halves were treated with ORO, both with and without prior 1,2-indanedione treatment by Patrick Fritz. Preliminary comparisons suggested that prolonged immersion in the non-polar 1,2-indanedione solution (approximately 5 seconds) caused subsequent ORO treatment to appear fainter, with less clearly defined detail (median grade of 1) compared to samples treated with ORO only (median grade of 2.5). When this time was reduced to 1 – 2 seconds, ORO fingermark development was not significantly worse than halves treated with ORO only. The 1,2-indanedione method did not appear to have any significant detrimental effect on fingermark halves subsequently treated with PD, compared to PD alone.

Fingermarks were halved and treated with the sequences ORO → PD and PD → ORO. While neither sequence appeared to outperform the other, the PD → ORO sequence seemed to produce slightly better contrast (Figure 3.9). Similar results have been reported for Beaudoin's ORO formulation, with the conclusion that PD → ORO was the superior method [159]. It has previously been reported that ORO treatment prior to PD treatment may cause greater destruction to the substrate during the maleic acid pre-treatment [159]. This effect was not observed in this study; however, fingermarks were collected on only one paper type here. Other substrates may be more reactive when immersed in maleic acid.

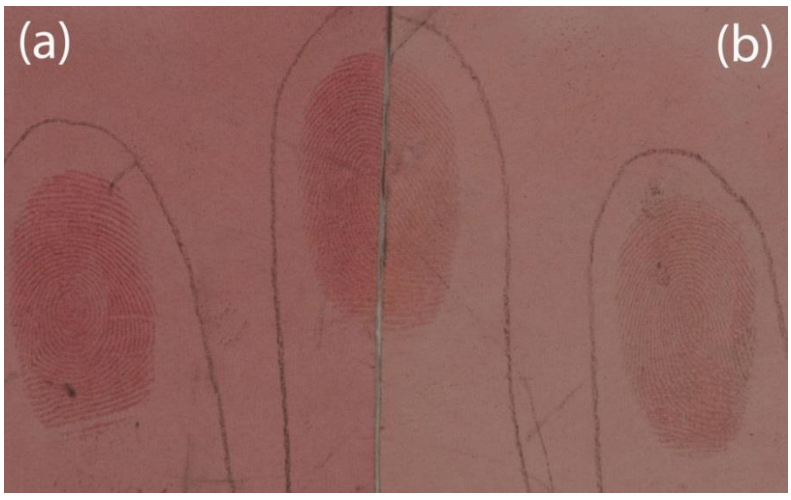


Figure 3.9: Fingermarks treated with a) PD → ORO sequence; and b) ORO → PD sequence

It was found that proceeding with PD treatment did not significantly improve the development quality of fingermarks that already showed good development with ORO. It did however improve the contrast between fingermarks and the substrate across all ORO-treated samples. A possible explanation for this is that ORO might act as a nucleation site for silver deposition [159]. Alternatively, it may simply be that the colouration provided by

ORO enhances the appearance of the fingermark on subsequent PD treatment. Though PD is often described as a lipid-sensitive reagent, some charged fingermark halves were only able to be detected when also using ORO, whereas fingermark halves treated with PD only showed little or no development. Conversely, in instances where a fingermark was only faintly developed by ORO (grade 0 – 1) PD enhanced the appearance of the ridge details (grade 2). This supports the hypothesis that PD targets compounds other than lipids [159, 164, 166, 167].

Though better quality fingermark development may be obtained using the reversed sequence of PD → ORO, there is a risk that PD may indelibly mar the surface of some paper types, preventing further treatment [159]. The PD working solution is highly reactive with some contaminants, and can blacken large portions of the substrate if sample pre-treatment is not conducted properly. Due to the destructive potential of this method, PD was placed last in the detection sequence, thereby following a logical application order of least destructive to most destructive methods [159, 175].

An example of a latent fingermark treated with the full sequence of 1,2-indanedione → ORO → PD is shown in Figure 3.10. The utilisation of these reagents forms a detection sequence for porous substrates that targets three separate groups of fingermark components: amino acids, 'fragile' lipids, and 'robust' water-insoluble compounds. This sequence was found to produce more well-developed fingermarks than any of the three individual treatments, and in the set order presented here there is no interference between treatments.

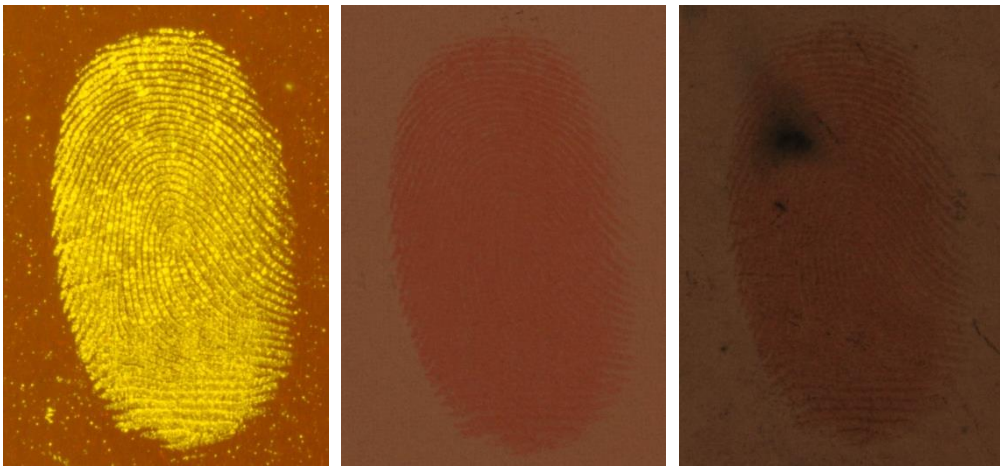


Figure 3.10: Fingermark treated with the full detection sequence (from left to right): 1,2-indanedione → ORO → PD

3.2.3.6 Shelf life of ORO reagent

Beaudoin *et al.* have reported that the methanol-based reagent may be stable indefinitely, as long as the solvent does not evaporate, and have reported the successful use of reagents at least 8 months old [177]. We believe that as with Beaudoin's formulation, ORO in propylene glycol can be used over an extended period of time, as long as there is no significant change in the colour of the solution, which should be a vibrant red with a noticeable pink/purple tinge. The histological stain on which it is based is purported to remain stable for one or two years. The fingermark development reagent provides consistent staining results over a period of at least several weeks, as long as significant precipitation of ORO does not occur. Briefly heating the reagent to 90 °C with constant stirring will redissolve the ORO precipitate and restore the staining properties of the solution. Solutions of up to 11 months old have been successfully used in this manner, but shelf life beyond this time has not been assessed.

3.3 Nile blue

3.3.1 Experimental

3.3.1.1 Sample collection

Unless otherwise stated, charged latent fingermarks were collected on white copy paper as described in section 2.6.4. For substrate investigations, samples were collected on a variety of porous and non-porous substrates. A complete list of the paper types examined is given in Table 3.2.

Table 3.2: Substrates examined throughout Nile blue investigations

Substrate	Manufacturer/Product
White copy paper	Fuji Xerox Professional
Post-it notes	Post-it
Thermal paper (unprinted and printed receipts; both sides of paper examined)	Officeworks, various unknown manufacturers
Patterned wrapping paper	Unknown manufacturers
Glass microscope slides	Esco Optics, USA
Plastic screw top lids	Unknown manufacturer
Ceramic crucible lids	Unknown manufacturer

3.3.1.2 Chemicals

Nile blue A (dye content ≥75 %; Sigma-Aldrich, USA), Nile red (Sigma-Aldrich, USA), methanol (Mallinckrodt Chemicals, USA), sodium hydroxide (≥97 %; Ajax Finechem, Australia), petroleum spirits 40 – 60 °C (APS chemicals, Australia) and hexane (Mallinckrodt Chemicals, USA) were all used as received and were of analytical reagent grade unless otherwise stated.

3.3.1.3 Preparation of reagent solutions

Preparation of Nile blue was based on the method described by Cain [228]. 0.05 g Nile blue A was dissolved in 100 mL deionised water with constant stirring. The solution was stored in a Schott bottle wrapped in aluminium foil.

Nile red stock solutions and modified working solution were prepared as described by Braasch *et al*. [187]. Preparation of all Nile red reagents is outlined in Table 3.3.

Table 3.3: Preparation of Nile red reagents

Solution	Reagent preparation
Nile red stock solution	0.025 g Nile red dissolved in 250 mL methanol
Sodium hydroxide stock solution	0.025 g sodium hydroxide dissolved in 250 mL deionised water
Modified working solution	230 mL Nile red stock solution added to 170 mL sodium hydroxide stock solution

3.3.1.4 Nile blue method development

For method development, a range of concentrations and treatment times were performed on quartered fingermark samples on white copy paper. Nile blue was prepared at concentrations of 0.001 – 1 g/100 mL. Samples were immersed in the reagent for 5 – 20 minutes. The optimised treatment method is described in 3.3.1.5.

3.3.1.5 Development of latent fingermarks using Nile blue

Fingermark samples were immersed in the reagent for 20 minutes. After treatment, samples were rinsed in a deionised water bath, and air-dried on paper towels at room temperature.

3.3.1.6 Development of latent fingermarks using Nile red

Sample treatment was carried out as described by Braasch *et al*. [187]. Samples were immersed in the working solution until fingermarks were visible (approximately 5 minutes),

then rinsed in deionised water for 5 minutes. Samples were then air-dried on paper towels at room temperature.

3.3.1.7 Illumination and photography

Samples were photographed using a Nikon D300 camera (details as outlined in section 2.6.7). Photographs were taken in both reflectance mode and luminescence mode (Table 3.4). Illumination in reflectance mode was achieved using incandescent light bulbs with no camera filter attachments. Illumination in luminescence mode was achieved using a Rofin Polilight® PL500 (Rofin, Australia), with an excitation wavelength of 505 nm and an orange camera filter attachment (550 nm barrier filter).

Table 3.4: Photographic conditions for reflectance and luminescence mode photographs

	Reflectance mode	**Luminescence mode**
Focal Length/ mm	60	60
Exposure Mode	Manual	Manual
White Balance	Auto	Auto
Shutter Speed/s	1/20	2
Aperture	f/11	f/11
Sensitivity	ISO 200	ISO 200

3.3.1.8 Instrumentation

Photoluminescence spectrophotometry

Photoluminescence was investigated using a Cary fluorescence spectrophotometer with a fibre optic probe attachment (Varian, Mulgrave, Australia). Fluorescence emission spectra were collected with excitation at 490 nm, excitation and emission slit widths of 5 nm.

High resolution mass spectrometry

High resolution mass spectra were obtained with a LTQ Orbitrap (Thermo Fisher Scientific, USA) and an electrospray ionisation source operated in positive ion mode. Samples were directly infused into the MS using a syringe pump at a flow rate of 5 µL/min. MS data were acquired in MS scan (85 – 1000 *m/z*) and in MS/MS scan mode (full MS^2 of 319.14 *m/z* under variable collision induced dissociation conditions). Full calibration of the LTQ Orbitrap XL in the 150 – 2000 *m/z* range was conducted with the positive ion calibration solution provided by Thermo Scientific. Optical lenses were optimised with a standard solution of Nile red prior to each measurement. For increased mass accuracy on the LTQ Orbitrap XL, a plasticiser interfering peak commonly present in solvents at *m/z* 214.0887 (n-butyl

benzenesulfonamide, $C_6H_5SO_2NH(CH_2)_3CH_3$, $[M+H]^+$ = 214.0896 *m/z*), was used for the lock mass function. Data was processed using Xcalibur QualBrowser 2.0.7 SP1 (Thermo Fisher Scientific).

3.3.2 Results and discussion

3.3.2.1 Preliminary investigations

Initial investigations were carried out to determine if Nile blue had potential to be utilised as a fingermark development reagent. Charged fingermarks on white copy paper were immersed in 1 % Nile blue for five minutes, following the histological staining method described by Cain [228], and rinsed briefly in deionised water. Samples were blotted and left to dry at ambient temperature on paper towels. The paper was stained a deep blue colour, while fingermarks were visible as darker blue-purple impressions. Little or no ridge detail could be seen under ambient lighting. Photoluminescence was observed when viewed under Polilight illumination at a wavelength of 505 nm, through an orange barrier filter (Figure 3.11).

Figure 3.11: Charged fingermark on white copy paper treated with 1 % Nile blue histological stain

3.3.2.2 Confirmation of Nile red formation

While it is accepted that Nile red is responsible for the fluorescence emitted by tissues stained with Nile blue reagent [180, 233-235], Nile blue A itself is often described as a photoluminescent dye. However, the excitation and barrier filter wavelengths that were used to examine Nile blue-treated fingermarks were similar to those used to examine

fingermarks treated with Nile red [187]. Several investigations were conducted to confirm which compound was responsible for producing photoluminescence in fingermarks.

10 µL aliquots of 1:1 linseed oil in hexane were pipetted onto white copy paper, dried, and treated with Nile blue. These exhibited photoluminescence under the same viewing conditions as treated fingermarks. This suggests that fingermark photoluminescence is due to the simple diffusion of Nile red into neutral lipids, as Nile blue A only interacts with acidic moieties, rather than triglycerides.

Photoluminescence emission spectra of latent fingermarks treated with Nile blue produced an emission maximum at approximately 560 nm with excitation at 490 nm (Figure 3.12). This is consistent with the viewing conditions required for fingermarks treated with Nile blue or Nile red [187]. When the Nile blue reagent was extracted into petroleum spirits, and the organic extract pipetted onto latent fingermarks, similar spectra were produced, further suggesting that Nile red is responsible for latent fingermark fluorescence.

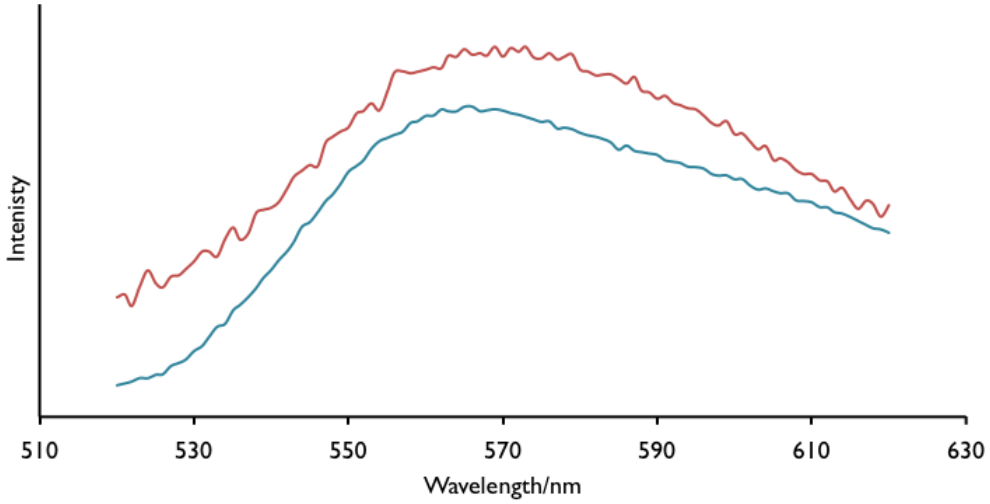


Figure 3.12: Fluorescence spectra of fingermarks treated with Nile blue (blue) and an organic extract of aqueous Nile blue (red) (λ_{ex} 490 nm)

Comparisons of high resolution mass spectra of the organic extract of Nile blue and a Nile red standard solution provided further evidence of Nile red as a component of the aqueous reagent. Collision induced dissociation experiments produced four characteristic fragment ions with the same accurate mass and similar relative abundance in both the extract and the standard (Figure 3.13). These results confirm that the photoluminescent component of the Nile blue reagent is Nile red.

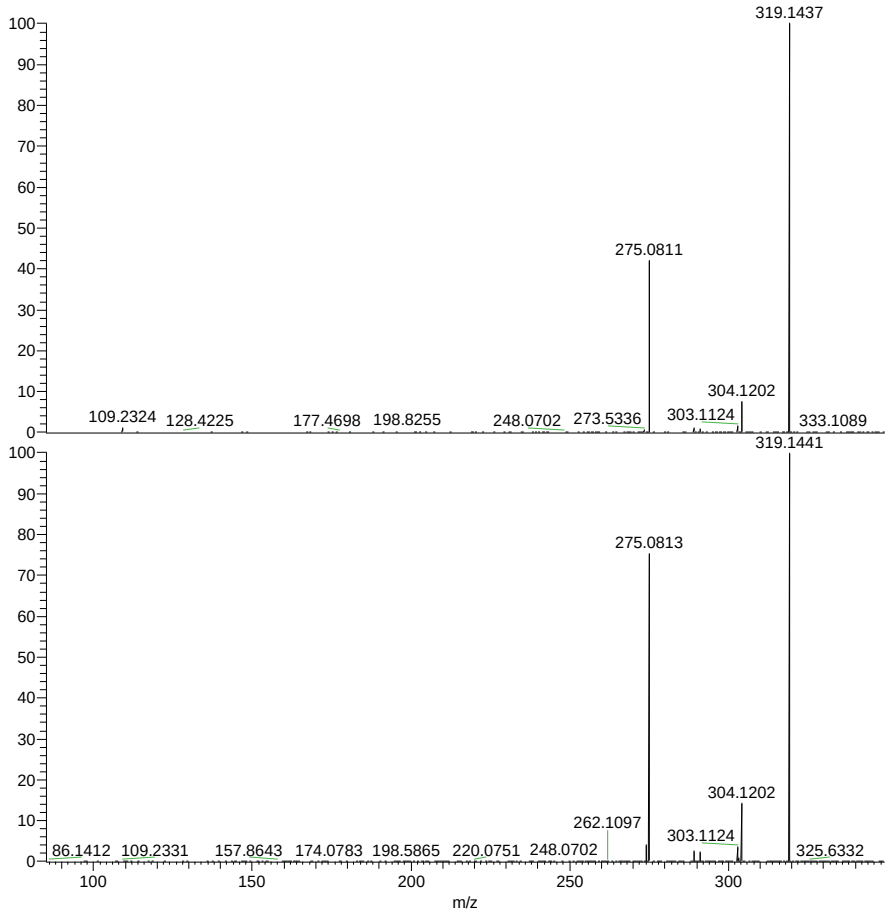


Figure 3.13: High resolution MS^2 spectra of a) Nile red standard solution; and b) organic extract of Nile blue

It should be noted that while Nile red is known to dissolve into neutral lipids, it is unclear what exact fingermark compounds are targeted by the Nile blue development reagent to produce fingermark photoluminescence. Linseed oil spots treated with Nile blue produced an emission maximum of 590 nm when excited at 490 nm. It is possible that the compositional differences between linseed oil (triglycerides) and latent fingermark residue, which contains a number of neutral lipids including triglycerides, wax esters and cholesterol, are responsible for this shift, as the photoluminescence emission and excitation maxima of Nile red are solvent-dependant [181, 239].

3.3.2.3 Method development

Concentration

Concentrations ranging from 0.5 – 1 g/100 mL all contained significant amounts of undissolved Nile blue A. Concentrations of 0.05 g/100 mL or lower were found to be sufficiently low that the Nile blue completely dissolved. Fingermarks treated with solutions of 0.002 g/100 mL or less still demonstrated photoluminescent ridge detail; however, the

substrate itself also became noticeably photoluminescent (Figure 3.14). While this does not significantly affect the quality of the developed fingermark itself, it does impact on contrast between the fingermark and the substrate, particularly with 'weak' fingermarks that contain low amounts of lipid material. It may be that Nile blue A has a quenching effect on Nile red photoluminescence that is necessary to achieve satisfactory contrast. Both dyes are absorbed into the paper substrate, where quenching of Nile red photoluminescence may occur, but only Nile red is absorbed into fingermark lipids, and is thusly protected from any quenching from Nile blue A.

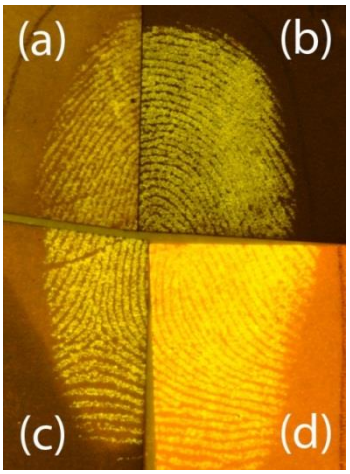


Figure 3.14: Fingermark developed with a) 0.5 g/100 mL; b) 0.05 g/100 mL; c) 0.005 g/100 mL; and d) 0.001 g/100 mL Nile blue

Based on these observations, 0.005 g/100 mL Nile blue A was determined to be the most suitable concentration for fingermark development, as fingermark development remained of a similar quality compared to higher concentration solutions, while utilising less reagent. Additionally very little dye remained undissolved, and good contrast between fingermarks and substrates could be achieved.

Development time

Initial experiments were carried out using a 5 minute treatment time as per the staining method described by Cain [228]. Immersing fingermarks in Nile blue for 20 minutes was found to produce stronger photoluminescence compared to shorter immersion periods of 10 minutes or less (Figure 3.15).

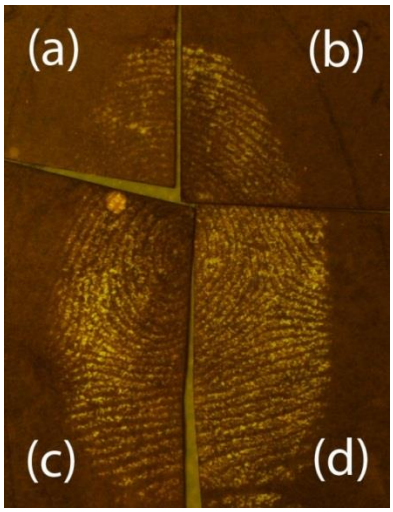


Figure 3.15: Fingermark treated with Nile blue for a) 5; b) 10; c) 15; and d) 20 minutes

3.3.2.4 Development of fingermarks samples exposed to water

Latent fingermarks halves were immersed in deionised water for one hour and left to dry on paper towels, before being treated with Nile blue. Comparisons with fingermark halves treated dry showed that immersion did not have any significant impact on Nile blue development or photoluminescence (Figure 3.16). To the author's knowledge, Nile blue is the second photoluminescent fingermark development method that can be successfully utilised on wetted, porous substrates, the first being the application of Nile red itself [187].

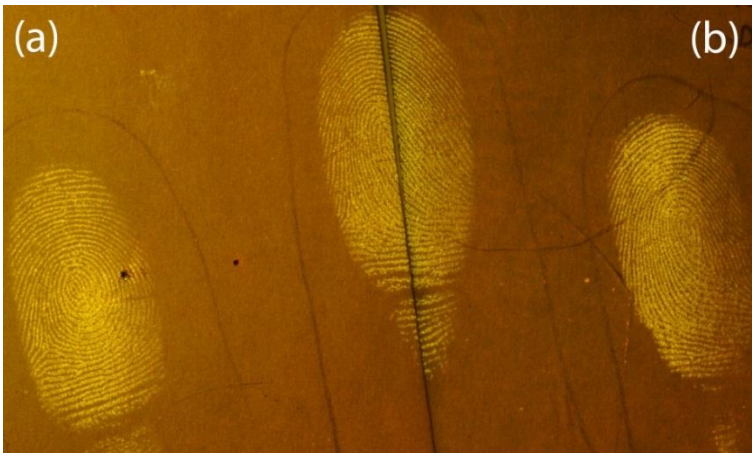


Figure 3.16: Fingermarks on copy paper treated with Nile blue after a) immersion in water for one hour; and b) no immersion

3.3.2.5 Substrate investigations

Fingermarks on a variety of substrates were treated with Nile blue in order to explore the versatility of the reagent (Table 3.4). Substrates included dark-coloured and patterned

substrates, which are known to be problematic for non-photoluminescent lipid-sensitive methods.

It was found that Nile blue was able to develop latent fingermarks on a variety of porous substrates, including dark-coloured paper and thermal paper receipts. Nile blue was less effective on adhesive substrates such as the adhesive strips of Post-it notes, as Nile red partitioned into the glue and produced intense photoluminescence (Figure 3.17).

Figure 3.17: Fingermarks on a purple Post-it note treated with Nile blue

Variable performance was observed on printed substrates such as wrapping paper and the reverse side of thermal paper receipts (Figure 3.18). Photoluminescent ridge detail was present on some portions of the text/patterns, but not on other parts. However, while some inks and dyes used in printing may supress photoluminescence, greater fingermark detail may still be observed using Nile blue than is possible with non-photoluminescent detection methods.

Figure 3.18: Fingermark on wrapping paper treated with Nile blue

In addition to the ability to develop latent fingermarks on both dry and wetted porous substrates, Nile blue was also found to develop latent fingermarks deposited on some glossy, non-porous surfaces (Figure 3.19). The visualisation of latent fingermarks on non-porous surfaces occurred in much the same manner as with porous surfaces, however on some non-porous substrates, treated fingermarks appeared blue and did not exhibit photoluminescence. In these cases, it appears that Nile blue A acts as a development reagent in conjunction with Nile red, likely reacting with the free fatty acids in fingermark residue to produce blue ridges. The combination of Nile blue A and Nile red in a single aqueous solution may therefore enable fingermark detection on a wider variety of surface types than is possible with Nile red alone, and this versatility represents a significant advantage in latent fingermark detection.

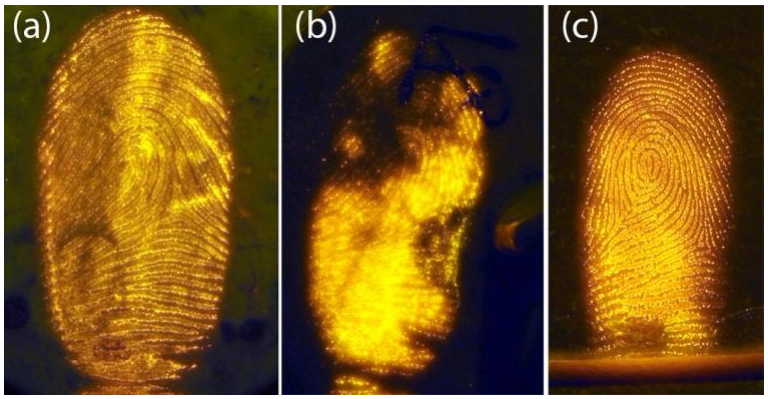


Figure 3.19: Latent fingermarks treated with Nile blue on (a) plastic lid; (b) ceramic crucible lid; and (c) glass microscope slide

3.3.2.6 Comparison to Nile red

A comparison to the Nile red reagent was performed on a depletion series of charged fingermarks. While the modified Nile red working solution was utilised, as it is reported to overcome solubility issues encountered with the original formulation [187], a thin red film was observed to form in the working solution, which if allowed to come into contact with the substrate may obscure developed fingermarks.

The two reagents performed comparably in terms of fingermark development quality (Figure 3.20). The contrast produced by Nile blue appeared to be slightly better than that of Nile red, due to the substrate photoluminescence produced by the latter treatment when viewed and photographed under identical conditions. As this comparison was only carried out on one brand of white copy paper, it is uncertain if this is typical of Nile red treatment. Due to time constraints, more extensive comparisons were not conducted.

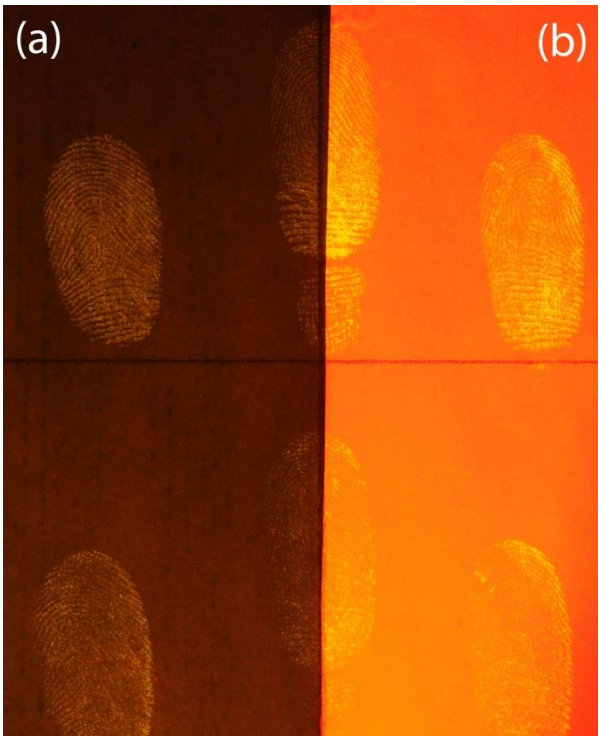


Figure 3.20: Latent fingermarks on white copy paper treated with a) Nile blue; and b) Nile red

3.3.2.7 Performance on older fingermarks

As Nile blue targets the same 'fragile lipid' fraction as ORO, it exhibits many similar limitations in regards to older fingermarks. While fingermarks up to 6 weeks old could be detected using Nile blue, samples older than 2 – 3 weeks demonstrated fainter or uneven photoluminescence compared to more recently deposited samples, as well as diffuse ridge detail (Figure 3.21). This is comparable to findings on the limitations of Nile red in the development of older fingermarks [187]. Additionally, Nile red partitions most effectively into lipids that are in liquid form [228, 232, 233]. As fingermark lipids oxidise, they become a solid, waxy residue, which may account for the diminished photoluminescence from older samples.

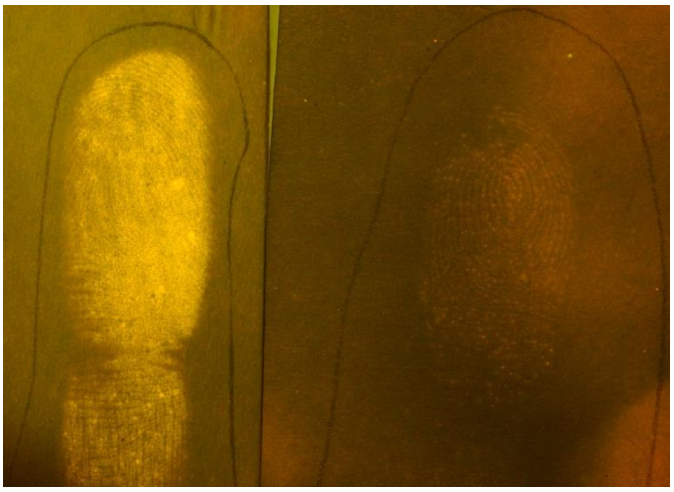


Figure 3.21: Fingermarks on white copy paper treated with Nile blue

6 weeks after deposition

3.3.2.8 Working life of Nile blue reagent

Further investigations are ongoing into the shelf life of Nile blue, as well as any changes in reagent performance which may occur over time. Nile blue can be reused several times, as long as the reagent maintains a vibrant blue colour. After several uses, the Nile blue takes on a paler, purple colour and while treated fingermarks are still readily visible, increased substrate photoluminescence is observed similar to results produced by low concentration Nile blue reagents. However, with the low cost of Nile blue reagent and ease of preparation, Nile blue can be justifiably discarded after use, and considering the risk of cross-contamination, this would be recommended in operational procedures.

3.4 Conclusions

This chapter has described investigations into two lipid-sensitive development reagents, one of which (Oil red O) will be used in conjunction with statistical methods to establish possible correlations between latent fingermark composition and detectability on porous substrates, as detailed in Chapter 4.

This novel ORO reagent is based on Chiffelle and Putt's histological formulation, modified to contain a significantly lower ORO concentration. ORO in propylene glycol shows promise as an alternative latent fingermark treatment to Beaudoin's methanol-based formulation, and performs comparably to both Beaudoin's ORO and PD on recently deposited, charged fingermarks. It is effective at developing recently deposited, lipid-rich fingermarks on a variety of porous surfaces, including samples that have been immersed in water. Results indicate the propylene glycol formulation performs equal to or better than PD on recently

deposited charged fingermarks, while PD is the superior technique for developing uncharged fingermarks. However, ORO in propylene glycol shows the same limitations as Beaudoin's ORO in regards to developing fingermarks on some substrate types and detecting aged fingermarks. The propylene glycol reagent was successfully incorporated into a detection sequence with 1,2-indanedione and PD.

Nile blue is presented for the first time as a development reagent for porous substrates. Nile blue exhibits potential as a cheaper and less hazardous alternative to Nile red that enables the development of fingermarks on both porous and non-porous substrates. The photoluminescent fingermarks produced by Nile blue provides a significant advantage over other lipid-sensitive development reagents in that fingermarks on dark or patterned substrates may be clearly seen. Additionally, the presence of the two dyes in one reagent enables the detection of fingermarks on a greater variety of substrate types than is achievable with any other lipid-sensitive reagent for porous substrates.

Chapter 4: Investigation into the effects of donor traits on the performance of lipid-sensitive reagents

4.1 Introduction

4.1.1 Donor influence on fingermark development

Variation in latent fingermark composition between individuals is easily demonstrated through the use of development reagents. By repeatedly collecting and developing fingermarks from a small population (e.g. the investigations described in Chapter 3), it can be observed that some individuals are consistently 'good' or 'poor' donors for target compounds such as amino acids or lipids [12, 27, 191, 240]. This so-called 'donor effect' is a well-known, but poorly understood phenomenon, and it is unclear as to why latent fingermarks from certain donors exhibit clear ridge detail, while those from others are barely visible when treated under the same operational conditions [109, 240]. Contributing factors are thought to include traits such as age, biological sex and diet, which have an influence on skin gland secretion rate and composition [11, 44, 98, 109, 191], as well as activities such as handling greasy food or washing hands with soap, which can impact on the amount of residue present on the skin ridges of the fingertips [10, 27, 109].

A preliminary study by Fritz *et al*. found significant variation between fingermarks collected from a large sample population, correlated to amino acid content, due to developed sample age, donor age and recent washing of hands [241]. More comprehensive investigations, utilising both amino acid- and lipid-sensitive reagents, could provide additional, complementary information regarding fingermark development as a reflection of fingermark composition. Charged fingermarks are often considered an unrealistic representation of latent fingermarks encountered in forensic investigations [15, 27, 191, 192]. There is anecdotal evidence to indicate that in some instances, latent fingermarks may still contain enough lipid material to produce visible ridge detail [242], warranting further study.

4.1.2 Comparison of Oil red O and physical developer

As described in Chapter 3, Oil red O (ORO) and physical developer (PD) perform very differently on both recently deposited and older charged fingermarks. ORO has been reported to outperform PD on recently deposited fingermarks and those up to 30 days old [159, 176], but is less effective on older fingermarks, which exhibit diffuse ridge detail [159]. PD can be more effective on fingermarks several weeks old than on those which have been recently deposited. Anecdotal evidence suggests that this may be due to the evaporation of volatile constituents that physically obstruct the electrostatic attraction of

silver particles to their targets [243]. Both reagents have been reported to perform more similarly on uncharged fingermarks [176].

As ORO produces superior fingermark development on recently deposited, charged fingermarks compared to PD, it has been suggested that ORO be incorporated into existing detection sequences for porous substrates, in conjunction with amino acid-sensitive reagents and PD [159, 238, 244]. For such a sequence to be considered viable for operational use, it must be demonstrated that the inclusion of ORO will enable a greater percentage of fingermarks to be detected. This has been achieved using charged fingermarks [159, 238, 244], but this is not necessarily representative of 'real' latent fingermarks, and ORO performance may be overestimated by these studies [245]. In order to gain a more realistic impression of the potential contribution of ORO to detection sequences, it must be determined what proportion of uncharged latent fingermarks could be expected to be recovered from a large donor population (n > 100), using both ORO and PD.

4.1.3 Assessment of fingermark development

The evaluation of the performance of a development reagent is a relatively unsophisticated process. Commonly employed assessment methods utilised in research generally categorise fingermark development along a scale ranging from 'good' to 'poor' ridge detail. The main issue with such ranking systems is their subjectivity; assessment of fingermark quality relies on human observation and, as such, is predisposed to bias stemming from an individual's own experience and personal notions as to what constitutes 'good' fingermark development [27]. It is known that these differences in personal opinion cannot be completely controlled by assessment protocols, as similar issues have been noted in the fingermark identification process [246]. These studies have indicated that experience and training play a pivotal role when an assessor encounters a fingermark that is incomplete or in some way distorted [246, 247].

The grading of treated fingermarks is usually done by a single individual, for the purpose of consistent method comparison or evaluation [27]. Although it is less common for the same fingermark to be assessed by two or more individuals, this could compensate for the subjectivity of fingermark assessment by enabling the use of mean or median fingermark grades. While employing a large group of experienced fingermark researchers would be an ideal approach to minimise bias, such an approach is rarely feasible due to individuals' workloads, and other commitments, as well as disparate geographical locations. A smaller,

localised group of graders would be more practical, provided that their performance can be safely assumed to accurately represent that of a larger group.

4.1.4 Aims

This chapter describes an investigation into variation of latent fingermark development as a function of the composition of the lipid fraction. Uncharged fingermarks were collected from a large donor population to compare the performance of ORO and PD on samples less than 36 hours old and 1 month old. A pilot study into the variability of fingermark quality assessment is also described. The work presented in this chapter was conducted as part of a large-scale collaboration with Patrick Fritz into the variability of fingermark development with amino acid- and lipid-sensitive reagents. As such, the data presented in section 4.2 is shared with Patrick Fritz as part of his PhD thesis (currently unpublished) [248].

4.2 Grader variation

Prior to the main investigation described in this chapter, a pilot study was conducted to assess the robustness of the grading method outlined in Chapter 2 against inter-grader variation, as well as intra-grader consistency, in fingermark assessment by several researchers from different research institutions, geographical locations, and varying familiarity with latent fingermarks. This was done to ascertain whether a small, localised group of fingermark graders could produce statistically reliable data, or whether a larger group would be necessary to overcome the subjective nature of the assessment method, despite the practical constraints of such an approach. The amino acid-sensitive reagent 1,2-indanedione was used to develop fingermark samples, as it is far more rapid than ORO or PD treatments.

4.2.1 Experimental

4.2.1.1 Chemicals

1,2-Indanedione (Optimum Technology, Australia), anhydrous zinc chloride (≥98 %; Sigma-Aldrich, USA), ethyl acetate (≥99.5 %; Univar Analytical, Australia), glacial acetic acid (≥99.7 %; Lab-Scan, Thailand), absolute ethanol (≥98 %; CSR Chemicals, Australia) and HFE-7100™ (20 – 80 % methylnonafluorobutyl ether, 20 – 80 % methylnonafluoroisobutyl ether; Novec, Australia) were all used as received and were of analytical reagent grade unless otherwise stated.

4.2.1.2 Preparation of reagent solutions

1,2-indanedione stock solutions and working solution were prepared as described by the Australian Federal Police (AFP) [168]. Preparation of all 1,2-indanedione reagents is outlined in Table 4.1.

Table 4.1: Preparation of 1,2-indanedione reagents

Solution	Reagent preparation
1,2-indanedione stock solution	4 g 1,2-indanedione dissolved in 450 mL ethyl acetate with 50 mL glacial acetic acid
Zinc chloride stock solution	8 g zinc chloride dissolved in 200 mL absolute ethanol
Working solution	50 mL 1,2-indanedione stock solution and 2 mL zinc chloride stock solution added to 450 mL HFE-7100™

4.2.1.3 Sample collection and storage

10 latent fingermark impressions (a depletion series of five 3-digit impressions from each hand) were collected from four donors on white copy paper (Fuji Xerox Professional). Donors had not washed their hands, consumed food or handled chemicals for at least 30 minutes before providing samples. Donors were asked to deposit impressions of the three middle fingers sequentially within printed templates as shown in Figure 4.1. The templates were divided into their 20 individual squares, to produce a total of 80 fingermark samples, and treated with 1,2-indanedione within 48 hours following deposition.

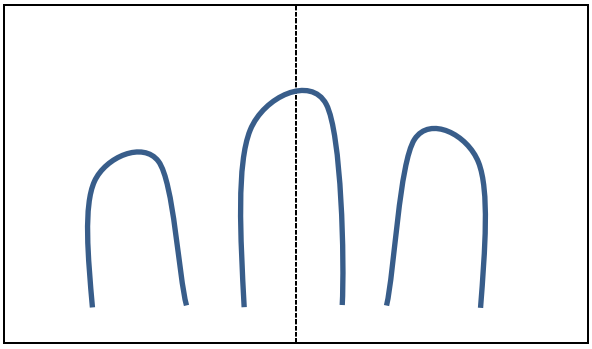


Figure 4.1: Schematic representation of finger placement for sample collection, showing two sample squares for a single 3-digit impression

4.2.1.4 Development of latent fingermarks with 1,2-indanedione

1,2-indanedione treatment was carried out by Patrick Fritz following the method as described by the AFP [168]. Samples were dipped briefly in the working solution and

allowed to air-dry. Samples were then heat-treated using an Elna laundry press for 10 seconds at 160 °C.

4.2.1.5 Illumination and photography of samples

Samples were photographed using a Nikon D300 camera mounted on a Firenze Mini Repro tripod and connected to a computer using Nikon Camera Control Pro Version 2.0.0. The samples were photographed in luminescence mode, with illumination and camera settings as described in Chapter 3.

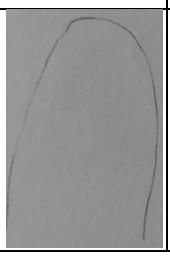
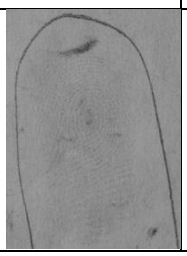
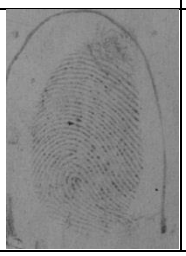
4.2.1.6 Data distribution and assessment of developed latent fingermarks

Sample images were assessed by eleven graders, who could be broadly classified into one of the following groups: experienced fingermark researchers (4), fingermark research students (3), and research students with no previous experience with fingermarks (4).

The consistency of each fingermark grader's performance in this study was examined from the grades assigned to replicated images. From the 80 sample images, 20 were randomly selected to be duplicated, producing a total of 100 images to be graded. The duplication of these images was done without the graders' knowledge. To reduce the effects of exhaustion and stress and to make the process less time-consuming, the images were distributed to the graders in 5 batches of 20 images each. This was implemented by numbering the images and then randomly assigning them to one of the 5 batches using a random number generator in Excel Professional Plus 2010 (Microsoft). The images were distributed to fingermark graders via an online cloud program, Dropbox (v.1.4.8).

Samples were graded using a 5-point system based on that used by the Home Office Police Scientific Development Branch, UK [211]. Detailed descriptions of each grade with example images were provided to the graders to reduce bias and encourage more consistent results (Table 4.2). The results were recorded and evaluated using Microsoft Excel Professional Plus 2010.

Table 4.2: The fingermark grading scale provided to the fingermark graders

Grade	0	1	2	3	4
Friction ridge detail development	No development	Signs of contact, but less than 1/3 of fingermark continuous ridges	1/3 – 2/3 of fingermark continuous ridges	More than 2/3 of fingermark continuous ridges, but not quite a 'perfect' fingermark	Full development; whole fingermark, continuous ridges
Contrast of ridge detail and background	No contrast	Poor contrast	Moderate contrast	Good contrast	Very good contrast
Photographic representation					

4.2.1.7 Data analysis

Data analysis of the 1,2-indanedione-treated images was carried out by Patrick Fritz. The median and mean grades for all images were calculated using Microsoft Excel. Statistical analyses were performed using SPSS version 2.0 (IBM). Non-parametric tests (Wilcoxon signed rank test and Mann-Whitney *U* test) were used in this chapter, as unlike parametric tests, these do not require the assumption that the data is normally distributed [249-251].

4.2.2 Results and discussion

4.2.2.1 Intra-grader variation

Consistent performance in fingermark assessment is crucial to the evaluation and comparison of fingermark development methods. If individual graders cannot be shown to assess fingermark development in a reproducible manner, the utilisation of subjective evaluation methods (i.e. grading scales) cannot be relied upon to produce meaningful data.

It was found that 172 (78.2 %) of the 220 image pairs assessed by the 11 graders received identical grades for each replicate (Figure 4.2). 48 (21.8 %) of the pairs showed a difference of 1 grade between the duplicates, and none were assigned a pair of grades with a difference of 2 or more.

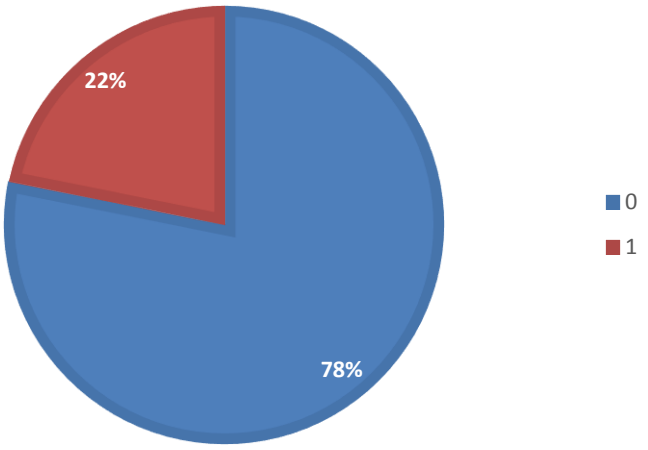


Figure 4.2: Differences between two grades assigned to duplicated image pairs

Of the duplicated image pairs that were assigned two different grades, half of these were graded inconsistently by at least two graders, indicating that these may be samples that are borderline (in between two grades) or otherwise difficult to categorise. Overall, it was found that very low and very high fingermark grades were the most easily reassigned to replicate images. The majority of disagreements were found to occur with images that were assigned a grade of 2 or 3 in at least one instance. These fingermarks, for example, may have shown good contrast and detail but also contained smudged regions, or did not exhibit continuous ridges (Figure 4.3). The more ambiguous quality of such fingermarks may have caused graders to rely more on their own individual impression of development quality, rather than adhering to the grading scale provided.

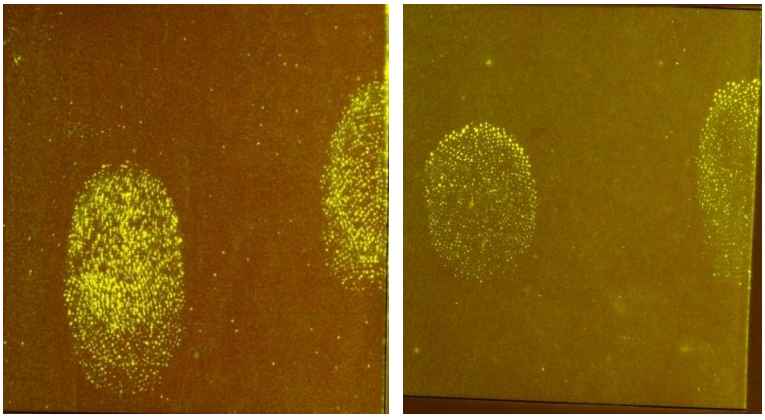


Figure 4.3: Examples of duplicated fingermark images graded inconsistently (left) and consistently (right) by the same individual

Wilcoxon signed rank tests were performed on the median and mean grades assigned by each grader to the duplicated image pairs as another means of investigating grader

consistency (Table 4.3). The Wilcoxon signed rank test can be considered as a non-parametric equivalent of a paired *t*-test [250]. Differences between the grades assigned to duplicate images can be considered significant if the absolute value of the calculated Z-score is greater than the critical value of 1.96 at a confidence interval of 95 % [252]. Similarly, if the calculated p-value is less than 0.05, the null hypothesis (i.e. that no significant difference exists) can be rejected at the same confidence interval. No significant differences were found between the grades assigned to duplicate image pairs by any grader (mean Z-score = -0.702, mean p-value = 0.53).

The relative prior experience each grader had with latent fingermarks appeared to have no significant impact on their ability to grade fingermarks consistently. These results indicate that this method of fingermark assessment, while subjective, can be considered as reliable and reproducible.

Table 4.3: Statistical values obtained from the Wilcoxon signed rank tests comparing the grades assigned by each grader for each duplicated pair

	Grader										
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
Median (original)	1	1	2	3	2	2	1	2	2	1	2
Mean (original)	2.15	2.1	2.35	3	2.3	2.4	2.1	2.3	2.25	1.95	2.4
Median (duplicates)	1	1	2.5	3	2	2	2	2	2	2	2.5
Mean (duplicates)	2.2	2	2.4	3	2.4	2.4	2.3	2.35	2.3	2.05	2.5
Std. dev. (original)	1.39	1.33	1.14	0.73	1.08	1.11	1.33	1.08	1.16	1.19	1.31
Std. dev. (duplicates)	1.44	1.26	1.11	0.92	1.11	1.11	1.38	1.09	1.26	1.05	1.24
Z-score	-0.58	-1.41	-0.45	0.00	-1.00	0.00	-1.63	-0.58	-0.45	-0.82	-0.82
p-value	0.56	0.16	0.66	1.00	0.32	1.00	0.10	0.56	0.66	0.41	0.41

4.2.2.2 Inter-grader variation

Agreement between graders, measured as how well the 11 grades assigned to each image coincided, is shown in Figure 4.4. For 42 images, the 11 grades given ranged over a range of one grade, and for a further 53 images, the assigned grades varied over a range of two. 3 images were given the same grade by all 11 graders. The remaining two images were graded the most inconsistently, with a total range of 3 grades assigned to these images. Similar to section 4.2.2.1, stronger agreement between all graders was more frequent when assessing fingermarks that exhibited very strong or very weak development (Figure 4.5). While the frequent and often widespread disagreements between graders appear to indicate that the grading scale is not a reliable tool to indicate of fingermark development

quality, these data only account for the absolute distribution of grades, rather than any consensus reached between the graders for each image.

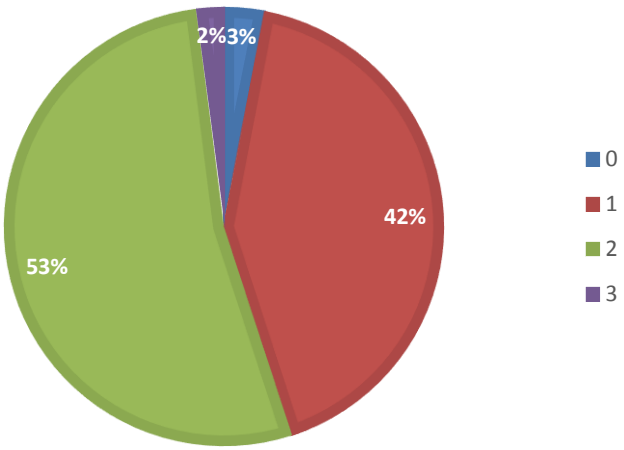


Figure 4.4: Ranges of grades assigned to each of the 100 treated fingermark samples

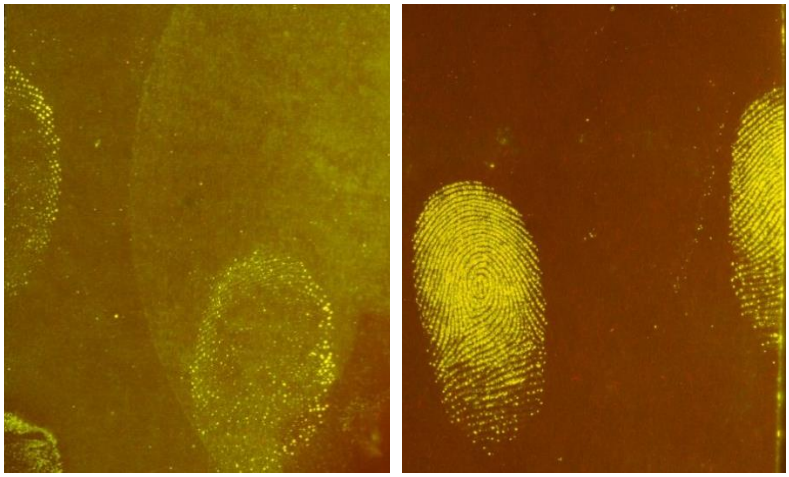


Figure 4.5: Examples of fingermark images unanimously assigned a grade of 1 (left) and 4 (right)

When the 11 grades assigned to each image were compared to the median, there was better agreement between all graders (Figure 4.6). Total agreement between any grade and the median occurred in 66.9 % of the 1100 total grades, and a difference of 1 grade occurred in 32.0 % of cases. In other words, 98.9 % of all grades provided differed by one or fewer from the median grade of the corresponding image. The remaining 1.1 % of grades differed by 2 from the median grade. No instance occurred where there was a difference of 3 or 4 grades to the median.

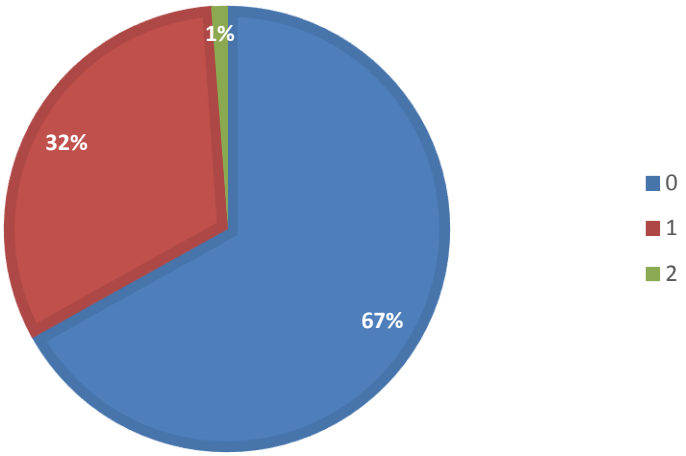


Figure 4.6: Differences between grades given to fingermark images and the corresponding median grade

While there was general agreement between all graders and the median, there were significant differences in graders' performances in how frequently they agreed with the median. Some graders assigned grades consistent with the median for 85 % of the fingermark images, for others, this rate was as low as 40 – 50 %. Similar results were found when graders' performances were compared to the mean grade for each image, rather than the median. The variation in grader performance therefore has a large impact on the results discussed above, particularly in regards to the mean grades. The median grade (the value separating the higher and lower 50 % of the grades assigned to each image) showed great similarity to the mode (i.e. the grade most frequently given), which is reflective of normal distribution of data. The median is therefore less affected by individual outliers than the mean. In general, there was strong agreement between the median and the mean grades. It should be noted that while some graders may not agree with the median grades as strongly as others, there was no significant difference in the consistency of their performances, as discussed in section 4.2.2.1.

While the performance of each grader appeared to have no correlation to institution or geographical location, graders with greater experience in fingermark research had a tendency to disagree with the median grades more often than the more inexperienced graders. It may be that with increased experience, these graders have formed their own standards for fingermark quality, and that this unconsciously influenced their performance even while using the provided grading scale. Conversely, the less experienced graders, some of whom were completely unfamiliar with fingermarks, may have been more likely to

rely almost solely on the grading scale as a guide. Similar trends have been observed in studies regarding the fingermark identification process [246, 247]. However, given that a pool of only 11 graders was used, only general trends can be drawn in this regard.

Inter-grader variation was further examined through the use of the intra-class correlation coefficient, which demonstrates the consistency between two or more objects of measurement (i.e. the fingermark graders) [253, 254]. The calculated intra-class correlation coefficient of 0.973 showed that there was very close agreement between the 11 graders. The lower and upper confidence intervals showed that assigned grades exhibited a correlation between 0.964 and 0.981 with 95 % confidence.

Based on these results, use of this grading scale appears to be a method that offers consistent and robust results for the assessment of fingermark samples and is therefore seen as a feasible approach for use in a pending large-scale donor study. Furthermore, it was found that a small subgroup of graders did not differ significantly in their assessment from the larger group, indicating that this approach may be used to avoid practical constraints in international collaborations. The use of median and mean grades assigned to a fingermark by multiple donors was therefore deemed an appropriate and consistent approach to overcome the subjectivity of fingermark assessment, either on its own or in combination with statistical methods.

4.3 Donor variation

4.3.1 Experimental

4.3.1.1 Chemicals and procedures

The materials and methods used throughout this chapter have previously been described in 2.6.5 and 2.6.6.

4.3.1.2 Sample collection and storage

Samples were collected by both Amanda Frick and Patrick Fritz. Uncharged latent fingermarks were collected from 148 donors on white copy paper templates, using the method described in 4.2.1.3, over an 18 month period. Donors were asked to briefly rub together the tips of the middle three fingers of each hand to evenly distribute any residue on the skin ridges. Donors were also asked to fill out a brief survey (Appendix 1.4) regarding traits or habits which may contribute to compositional variation. A summary of the donor population demographics is outlined in Table 4.4.

Table 4.4: Summary of donor information

Sex	**Male (*n*)**	**Female (*n*)**
	77	71
Age(years)	**Under 25 (*n*)**	**25 and over (*n*)**
	69	79
Recent washing of hands	**Yes (*n*)**	**No (*n*)**
	60	88
Recent food handling	**Yes (*n*)**	**No (*n*)**
	79	69
Washing of hands since handling food	**Yes (*n*)**	**No (*n*)**
	55	93
Recent use of skin products (within 12 hours)	**Yes (*n*)**	**No (*n*)**
	62	86
Recent use of skin products (within 24 hours)	**Yes (*n*)**	**No (*n*)**
	84	64
Recent handling of other dirty/greasy substances	**Yes (*n*)**	**No (*n*)**
	21	127
Total	**148**	

One depletion series from each donor was randomly assigned to be developed with the ORO → PD sequence discussed in Chapter 3. Each sample was divided into half, and the left half of each sample was treated within 24 hours of collection, while the right half treated after 30 days in storage, under the conditions described in Chapter 2.

4.3.1.3 Fingermark development

Sample treatment with ORO and PD was carried out as described in Chapter 2 by Amanda Frick. After development with ORO, samples were left to dry overnight, before being photographed. On the following day, the samples were treated with PD.

4.3.1.4 Photography of developed fingermarks

Samples were photographed post-treatment as outlined in Chapter 2 by Amanda Frick and Patrick Fritz.

4.3.1.5 Data distribution and assessment of developed latent fingermarks

Sample images were assessed by five of the graders (1 fingermark researcher, 1 experienced student and 3 students unfamiliar with fingermarks) who had participated in Section 4.2. The images were distributed and assessed as outlined in 4.2.1.6.

4.3.1.6 Data analysis

Data analysis was performed as outlined in 4.2.1.7 by Amanda Frick.

4.3.2 Results and discussion

4.3.2.1 Intra- and inter-grader variation

41 randomly selected images of ORO and PD-treated samples were duplicated and redistributed to the fingermark graders, to ensure consistency in graders' performances, as discussed in section 4.2.2.1. It was found that 163 (79.5 %) of the 205 image pairs were assigned the same grade on both occasions (Figure 4.7), similar to the results obtained in the grading study. This engenders confidence in the reliability of the data produced by the five graders. Of the duplicated images that were graded inconsistently, only two were assigned disparate grades by more than one grader. The uneven substrate staining caused by ORO and PD treatment in some instances may account in part for discrepancies between replicate grades, as these may obscure, or be mistaken for, fingermark development.

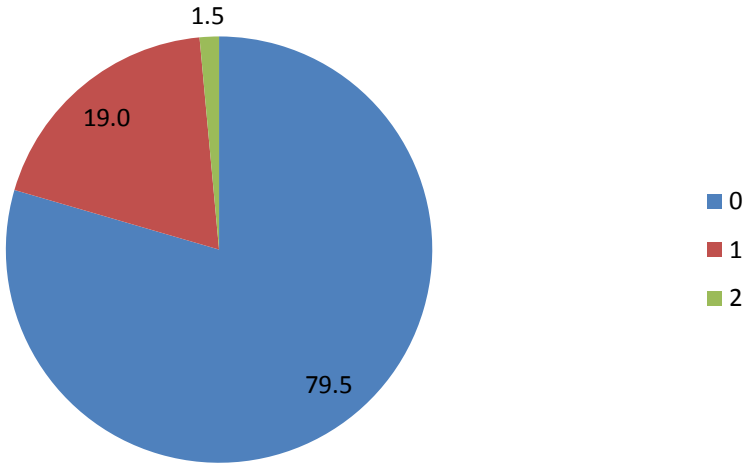


Figure 4.7: Differences between two grades assigned to duplicated images

Wilcoxon signed rank tests were performed to compare the differences between grades given to duplicate image pairs by each grader (Table 4.5). No significant differences were found between the two grades assigned to each pair by any grader. The calculated intra-class correlation coefficient of 0.921 further demonstrates that there was good agreement between the 5 graders. The lower and upper confidence intervals showed that assigned grades exhibited a correlation between 0.883 and 0.944 with 95 % confidence.

Table 4.5: Statistical values obtained from Wilcoxon signed rank tests comparing grades assigned by each grader to duplicate image pairs

Grader	1	2	3	4	5
Median (original)	1	1	0	1	1
Median (duplicate)	1	0	0	1	1
Mean (original)	0.73	0.95	0.59	0.82	1.27
Mean (duplicate)	0.71	0.78	0.39	0.66	1.02
Std. dev. (original)	0.71	0.95	0.59	0.82	1.27
Std. dev. (duplicate)	0.64	0.96	0.59	0.72	1.01
Z-score	-.58	-.17	-1.00	-.38	-1.81
p-value	0.56	0.87	0.32	0.71	0.07

4.3.2.2 Comparison of PD and ORO performance

A total of 2960 grades were assigned to 740 fingermark images by five graders. Marked differences between ORO and PD performance were evident at both time points examined in this study, and this was reflected in the distribution of the grades, as shown in Figure 4.8. Approximately 70 % of the grades assigned to PD-treated samples, regardless of sample age, were of 1 or greater, indicating some ridge development. ORO performance appeared to be significantly worse in comparison, with only 37 % and 11 % of grades assigned being of 1 or greater for samples treated within 36 hours of deposition and after 1 month, respectively. Grades of 1 comprised 79 % of grades greater than 0 assigned to ORO-treated samples, regardless of sample age. The poor overall performance of ORO, in contrast to previous reports [175, 176], can be attributed to a minimal amount of lipid material in these uncharged samples. These results furthermore support the hypothesis that the two reagents target separate groups of water-insoluble compounds, which exhibit differences in stability, as PD performance was less affected by sample age than that of ORO [159, 186]. The relative performances between PD and to ORO in this study are consistent with previous reports that PD provides superior fingermark development on samples greater than approximately 1 month old, as discussed in Chapter 3.

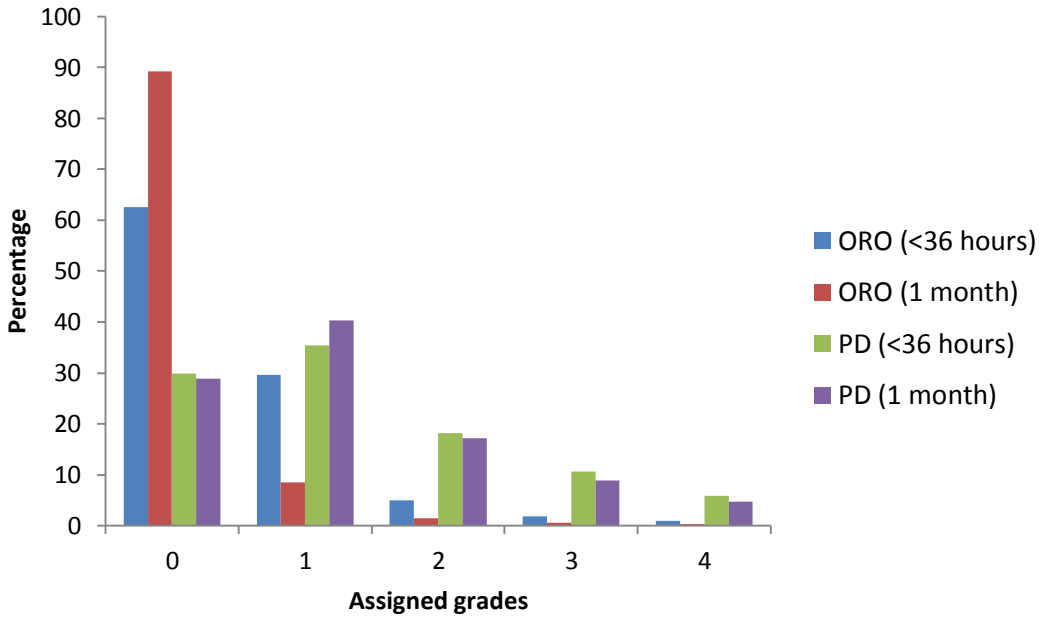


Figure 4.8: Distribution of all grades assigned to developed samples by five graders

Though ORO was shown to be unable to match PD performance on uncharged fingermarks, this does not necessarily mean that ORO ought to be discounted as a potential method for operational use. The PD working solution is often regarded has having a tendency to be destructive, and may produce rapid and uncontrolled deposition of silver across the entire substrate, resulting in a uniform grey-black appearance [121, 168, 189, 190]. This may be due to the incomplete removal of carbonate fillers from the substrate, the stability of the redox solution, or simply 'bad luck', as blackening of the substrate can occur even when the procedure is performed correctly and with newly prepared reagents. In this study, approximately 19 % of PD-treated samples were ruined in this manner, with a further 38 % exhibiting patchy deposition of silver that in some cases obscured part of the fingermark. In such instances, any previous application of ORO might still enable the recovery of some ridge detail.

As only a minority of ORO-treated images exhibited any ridge detail, these samples were considered unsuitable for any exploratory statistics into the effects on donor traits or sample age on fingermark development, as the resultant datasets were deemed too small. Consequently, only the grades assigned to PD-treated images were used to determine any statistically significant differences in fingermark detectability.

The Wilcoxon rank test was performed to determine the significance of the differences in the grades assigned to PD-treated images of different ages seen above (Table 4.6). It was

found that there was no significant difference in the median grades assigned to images of fingermarks treated within 36 hours of deposition, and those treated 1 month after deposition. This suggests that the overall effects of sample age on PD efficacy are negligible within the time period investigated in this study.

Table 4.6: Statistical values obtained from the Wilcoxon signed rank test comparing median grades given to PD-treated samples of different ages

Age of sample	<36 hours old	1 month old
Number of donors	148	148
Median	1	1
Mean	1.27	1.20
Standard deviation	1.17	1.10
Z-score	0.70	
p-value	-0.39	

4.3.2.3 Inter-donor variation

Mann-Whitney *U* tests were performed on the mean and median grades assigned to each image, with fingermarks developed within 36 hours and those treated after 1 month treated as separate datasets. The Mann-Whitney *U* test, also known as the Wilcoxon rank sum test, can be considered as a non-parametric equivalent to the independent *t*-test, and is used to compare two independent groups (e.g. male and female) based on a single variable [255].

The results of these tests indicated that there were significant differences in the grades assigned to PD-treated images related to donor sex ($Z = -2.52$, $p = 0.01$), age ($Z = -2.19$, $p = 0.03$) and the use of skin products within 12 hours prior to sampling ($Z = -3.33$, $p = 0.0009$), as shown in Table 4.7. Comparisons of these results with the grades assigned to the sample images showed that fingermarks from female donors, donors over the age of 25, and donors who had recently used skin products were given higher mean grades than samples from male donors, donors under 25 years old, and donors who had not used skin products, respectively. No significant differences in PD development were attributed to other factors such as the recent washing of hands or food consumption.

Table 4.7: Statistical values obtained from Mann-Whitney *U* tests of median grades given to samples treated with PD within 36 hours, as a function of donor traits

	Sex		Age (years)		Recent washing of hands		Recent food handling		Recent skin product use (12 hours)	
	Female	Male	Under 25	Over 25	Yes	No	Yes	No	Yes	No
Donors (*n*)	71	77	69	79	60	88	79	69	62	86
Median	1	1	1	1	1	1	1	1	1	1
Mean	1.44	1.05	1.07	1.38	1.08	1.34	1.27	1.20	1.56	1.00
Standard deviation	1.05	1.07	1.02	0.94	0.94	1.15	1.02	1.15	1.05	1.04
U score	2105.5		2181		2362.5		2561.5		1846.5	
Z-score	-2.52		-2.19		-1.19		-0.66		-3.33	
p-value	0.01		0.03		0.24		0.51		0.0009	

Further Mann-Whitney *U* tests were performed on subsets of this data to compare the influence of biological sex and age amongst donors who used skin products and those who did not, and vice versa. The obtained results, provided in Appendix 2, indicate that the perceived differences between male and female donors may in fact be related to the use of skin products, i.e. female donors who use skin (particularly cosmetic) products, rather than compositional differences in skin secretions inherent to sex. No significant differences in fingermark development were evident as a function of donor sex when the data was divided into two subsets based on donors' recent use of skin products. Interestingly, the use of skin products appeared to contribute significantly to variation in fingermark development amongst male donors, but not female donors.

Significant differences in fingermark development attributable to donor age were found amongst a subset of donors who had not used skin products, with sample from donors aged 25 years and over exhibiting higher mean grades. When donors under 25 years of age were divided into further subsets based on skin product use, significant differences were again seen, with skin product use found to be related to increased mean grades. No significant differences were found within subsets of donors over 25, based on skin product use, or among donors who used skin products as a function of age. The differences in PD development based on age can be attributed to the differences in sebum secretion rate and composition between adults and children [38, 44, 69, 75, 98, 100, 101], who comprised a large proportion of donors under the age of 25. As the use of skin products was found to have a dramatic influence on fingermark development, it can be inferred that the differences between donors under 25 years of age based on skin product use, and the lack of age-based differences amongst donors who used skin products, are due to the

introduction of extraneous PD targets in the form of skin product components. The presence of such compounds may act to mask the deficiency of endogenous target compounds in the fingermarks of younger donors.

No statistically significant differences between any donor factors were found for images of fingermarks that had been treated 1 month after deposition (Table 4.8). However, as shown in 4.3.3.2, there is little difference in the performance of PD on samples of these two ages. As there is no significant inter-donor variation amongst samples treated after 1 month, this may indicate a 'levelling-out' of compositional variation in PD target compounds as a function of time. For example, there may be two competing mechanisms of compositional changes, such as the degradation of skin product components, leading to decreased PD development, and the hypothesised changes in the physical properties of fingermark residue that may improve PD development [243]. Further studies utilising analytical chemical methods are required to determine the exact nature of these compositional changes.

Table 4.8: Statistical values obtained from Mann-Whitney *U* tests of grades given to samples treated with PD after 1 month, as a function of donor traits

	Sex		**Age (years)**		**Recent washing of hands**		**Recent food handling**		**Recent skin product use (12 hours)**	
	Female	**Male**	**Under 25**	**Over 25**	**Yes**	**No**	**Yes**	**No**	**Yes**	**No**
Donors (*n*)	71	77	69	79	60	88	79	69	62	86
Median	1	1	1	1	1	1	1	1	1	1
Mean	1.29	1.13	1.17	1.24	1.15	1.25	1.34	1.06	1.31	1.14
Standard deviation	1.13	1.30	1.07	1.02	1.09	1.00	1.01	1.06	1.17	0.94
U score	2563.5		2561.5		2364		2254		2529.5	
Z-score	-0.69		-0.67		-1.20		-1.92		-0.56	
p-value	0.49		0.50		0.23		0.054		0.57	

4.4 Conclusion

The pilot study showed that the fingermark grading method used in research at Curtin University is a robust and reliable method, insofar as subjective assessment is concerned, for obtaining data from several graders. When instructed in detail regarding the fingermark assessment protocol, graders were found to often perform in agreement with each other. Furthermore, it was demonstrated that grader performance remained consistent when assigning grades to a large number of fingermark images.

Examination of the grades given to fingermarks treated with ORO and PD both within 36 hours of deposition and after being stored for 1 month revealed significant differences in the relative performance of these two reagents. PD was shown to perform better than ORO, with 70 % of samples showing some ridge development, regardless of age, while ORO detected 37 % and 11 % of recently deposited and stored fingermarks, respectively. These results are consistent with the hypothesis that these reagents target different groups of water-insoluble fingermark components. The instability of the PD working reagent was also demonstrated, with 57 % of treated samples exhibiting moderate to severe substrate blackening.

Statistical analyses of the PD-treated samples found significant differences in fingermark development of recently deposited samples related to donor age and sex, as well as recent use of skin products. Further examination of subsets of the donor population based on these traits indicated that some of these differences may exist as a function of the latter. Samples treated with PD after 1 month showed no significant variation attributable to the donor traits investigated.

Chapter 5: Analysis of the initial composition of latent fingermark lipids by gas chromatography-mass spectrometry (GC-MS)

5.1 Introduction

As indicated in the previous chapters of this thesis, there are many variables which can contribute to latent fingermark composition. 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 [15, 161]. 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 [11, 44, 98, 102]. 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 fingermark composition such that the analysis of latent fingermark composition may allow these traits to be inferred [11, 12, 15, 44].

There is a need for a more extensive understanding of fingermark chemistry for the further development of latent fingermark detection capabilities. An investigation such as that described in Chapter 4 can establish correlations between donor characteristics and fingermark development, and therefore enable inferences about the differences in fingermark composition, but is limited in revealing more concrete information. Analytical techniques, which are more selective and far more sensitive, are much better suited to establishing any trends or discrimination between samples. To date, gas chromatography-mass spectrometry (GC-MS) is one of the most utilised methods for studies into latent fingermark composition, particularly of the lipid fraction [25, 127], as these compounds have been reported to differ quantitatively as a function of donor age [11, 44]. 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 [4, 12, 15, 41, 256].

5.1.1 Application of GC-MS to sebaceous lipids

GC-MS is one of the most widely used quantitative and qualitative analytical techniques in the separation and characterisation of complex mixtures [49, 257, 258]. It is used extensively in a number of biological and medical applications [49, 258], as well as many forensic applications, including toxicological analyses, and examination of debris recovered from arson scenes, paint and inks [259].

Much of the characterisation of skin surface lipids was conducted predating the widespread use of GC-MS, using separation procedures such as fractional distillation [260], thin-layer chromatography [37, 59, 63, 64, 80, 82, 100, 105, 261], and GC coupled to thermal conductivity or flame ionisation detectors (FID) [49, 68]. The first application of GC to the simultaneous analysis of several major sebaceous lipid classes (free fatty acids, squalene, cholesterol and wax esters) was reported by Haahti *et al.* in 1962 [262]. Many investigations before and since have typically focused on a specific lipid class, such as fatty acids or wax esters, rather than multiple compound types [54, 66, 67, 82, 88]. The greater sensitivity and selectivity possible with mass spectrometry detectors has since supplanted the above techniques. GC-MS analyses of human skin surface lipids have enabled the identification of hundreds of species, and provided detailed information regarding the unusual chain structures of these lipids (described in Chapter 1) [49, 84].

GC-MS is most effective with thermally stable, volatile analytes. Large, neutral lipids such as wax esters and triglycerides can be problematic to analyse due to their less volatile natures, as well as difficulties in ionisation of these compounds [50, 262, 263]. Lipids may be made more amenable by derivatisation to more volatile esters, either of the intact molecules or their hydrolysis products [49, 67, 69, 79, 84, 257, 264]. Derivatisation has the further advantage of avoiding analyte dimerisation [120]. Analysis of hydrolysis products presents a particular problem in characterising analyte structure, as while the structures of the constituent fatty acids and alcohols can be determined, including positioning of methyl branches and double bonds, their arrangement within the parent molecule cannot [50, 66, 84, 87, 88, 265].

5.1.2 GC-MS analysis of fingermark lipids

The first reported GC studies into the lipid composition of fingermarks were carried out by the Atomic Energy Research Establishment, UK in the 1970s [29, 120, 132, 133]. The impetus behind these investigations was to obtain a better understanding of potential reagent targets, namely free fatty acids, that might enable the development of fingermarks on substrates exposed to water [120]. These initial studies confirmed that the primary source of fingermark lipids is from touching sebum-rich areas of skin such as the face, rather than from other postulated sources such as the epidermis of the fingertips, or the migration of sebum down the arms to the hands [132]. When hands had been cleaned and wrapped in plastic bags, it was found that no additional lipid material was produced, however when normal touching of the face was permitted, fingermarks showed normal lipid profiles once more [132]. Additionally, GC-FID of latent fingermarks found the same

major lipid compounds as reported in sebum. It was also demonstrated that the amount of residue transferred from the fingertips is heavily dependent on substrate type [120]. Fingermarks deposited on porous substrates were typically found to contain more total material than those deposited on nonporous surfaces due to their absorption into the substrate. It was noted that makeup worn by female donors often became incorporated into latent fingermarks, an issue which often affects fingermark studies [11]. The presence of cosmetics and other skin products was found to introduce many low molecular weight compounds into fingermark residue, complicating analysis in this mass range [133].

Despite a lack of detailed knowledge regarding fingermark lipid composition, there appears to have been no pressing impetus to investigate any further for several decades. Studies into the factors affecting fingermark composition, such as donor age, were not carried out until the mid-1990s [158]. It had been noted that in certain circumstances where an adult's fingermarks could be detected several days after deposition, those left by young children could not [44, 102]. This 'vanishing' of children's fingermarks was encountered in an investigation of abduction. Despite witness testimony placing the child victim in the suspect's car, only the suspect's fingermarks were found on the interior surfaces [44, 158]. This indicated that children's fingermarks evaporated at a significantly faster rate compared to those of adults [102]. Pilot investigations found that latent fingermarks left by pre-pubescent children on non-porous surfaces were often unable to be developed 24 hours after deposition, whereas those left by adults were more durable [44, 102, 158]. Based on these results, Bohanon *et al*. recommended that any items on which a child's fingermarks might be found be processed as soon as possible and stored in cool conditions [102]. Following these preliminary results, a study was conducted by Buchanan *et al.* using GC-MS to investigate the compositional differences between children's and adults' fingermarks [44, 102]. It was found that the fingermarks of prepubescent children contained far fewer non-volatile lipids, such as wax esters, which accounted for the rapid 'disappearance' of children's fingermarks under conditions which did not impact the detection of adults' [102]. Mong *et al*. conducted a similar study, which aimed in part to identify the compositional differences between children's and adults' fingermarks, while determining how pubescent individuals compared [11]. The results of the analysis of fresh fingermark samples were similar those of Buchanan *et al*., regarding comparisons between adults and prepubescent children. In both studies, significant variation between individual donors was observed, and it was postulated that this might form the basis of a method of discrimination for the purposes of criminal investigations [11, 44].

Initial investigations into differences due to biological sex were carried out by Asano *et al*. as a possible means of determining individual traits, in circumstances where the pattern of a fingermark proved unsuitable for identification [41]. It was noted that while the relative peak areas of palmitic, palmitoleic and oleic acids were on average greater in male donors than females, no statistically significant differences were observed. Similarly, Croxton *et al.* were unable to differentiate samples based on donor traits, though it was pointed out that this was not an unexpected result, as the quantity of any one fingermark constituent is likely to be affected by a combination of factors, rather than a single trait [15].

As the majority of latent fingermark lipids are derived from sebum, it follows that analytical considerations applicable to studies of the former are also relevant to the latter. Due to the non-volatile nature of many lipid compounds present in sebum, most GC-MS analysis of latent fingermarks has employed derivatisation [10, 11, 15, 44]. In addition to the issues described above, this additional step in sample preparation introduces further variability, such as incomplete derivatisation and the generation of side products [11, 263, 266]. These additional variables not only affect reproducibility within a study, but can make it difficult to compare results derived from different studies, as all have used different preparation procedures [11, 50]. As silylation leaves sample compounds vulnerable to hydrolysis if stored improperly, Croxton *et al*. used ethyl chloroformate, to enable simultaneous detection of fatty acids and amino acids [32]. While this method produces more stable derivatives, it is limited in focus regarding the number of compound classes that were reported, compared to other studies of both derivatised and underivatised samples [11, 14]. Another difficulty of this approach is that derivatised standards must be prepared to enable identification of sample compounds [10], which can complicate analyses where the identities of constituents are poorly understood, or standards are simply not available.

The first reported separation of a variety of fingermark lipids without derivatisation was reported by Asano *et al*. in 2002 [41]. Most of the major fingermark lipid classes, including free fatty acids, squalene, cholesterol and wax esters, were detected in this manner. A similar method has been employed in research at the University of Lausanne, enabling the identification of over 50 wax esters [12-14]. The main advantages of this approach are simplicity, minimal sample preparation and time-effectiveness when analysing a large number of samples, as well as being able to detect a wide variety of lipid types simultaneously [132]. It can, however, be limited in detection of wax esters and triglycerides due to their low volatilities at the maximum temperature limits of the columns used [84, 86].

In addition to the above, there are further experimental considerations which are unique to fingermark samples. Similar to the issues regarding fingermark development investigations discussed in Chapter 2, the collection of latent fingermarks varies widely between analytical studies. Porous substrates are the most commonly utilised, though non-porous substrates have also been used, while others have sampled directly from the fingertips by solvent extraction [10, 11, 44]. The former is the more widely used collection method, as it enables investigations into the degradation of fingermark residue, being obviously more realistic of latent fingermarks left at crime scenes. Additionally, there is a possibility that direct solvent application could extract lipid material from within the epidermis as well as from the skin surface [120], and this is indeed a method used in sampling of total skin lipids in dermatological research [267]. Many other factors including temperature, fingertip pressure and duration of contact can also have an effect on the quantity and composition of the material in a latent fingermark [11].

Exogenous contamination is often a problem with fingermark collection, particularly in the case of charged fingermarks. Substances such as cosmetic products, soap, and other skin care products can contribute significant amounts of fatty acids and cholesterol, as well as a number of other compounds not endogenous to human skin [10, 117, 133]. Several investigations into human skin lipid and fingermark composition have reported complications arising from the frequent contamination of samples by skin products, even when protocols were designed to prevent donors from using such products during the study [11, 52, 268]. Compounds derived from skin products may be detected in samples collected up to two weeks since their last application, indicating their persistence on the skin surface, though this may also be due in part to donor non-compliance [11, 52, 268]. It has been postulated that exogenous compounds might play a useful role in profiling of fingermark composition [52, 269, 270].

Lastly, 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 [10, 12, 14, 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 [15, 256]. 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 [11, 44].

5.1.3 Principal component analysis

The volume of multivariate data generated by large-scale analytical studies requires multivariate statistical analysis in order to derive meaningful information from the dataset [12, 15, 250]. One of the most widely used multivariate statistics methods is principal component analysis (PCA) [271]. 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 [271-274]. 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) [271, 273, 275-277]. The first PC explains the largest percentage of variance within the original dataset, and each subsequent PC describes a decreasing value of the remainder [272, 274, 275, 278]. Generally, only the first few PCs need to be examined to account for the vast majority of the variance within the original dataset. These PCs may then be used to construct a scores plot: a 2- or 3-dimensional visualisation of patterns and relationships within the dataset that may not be discernible at first glance [250, 274-276, 279]. Further interpretation of the data is achieved through comparison of the scores plot with the loadings plot, which indicates the variable(s) in the original dataset which have the greatest influence on each PC [140, 276, 280, 281]. PCA is often used in exploratory data analysis and in the construction of predictive models in conjunction with linear discriminant analysis (LDA) [277-279]. PCA has also been employed as a data processing step to enhance infrared images of latent fingermarks, though as mentioned in Chapter 1, such studies have focused on chemical imaging as a detection method rather than as an analytical tool [134, 135, 140].

There has been an increased demand for more objective methods of data interpretation in forensic analyses [282], as many comparisons between data from known and unknown samples are often carried out by visual evaluation, which is highly subjective and limited to very small datasets [274, 276-278]. As such, multivariate statistics and other chemometric approaches are finding increasing relevance to these disciplines [276, 278]. PCA has demonstrated potential use in several areas of forensic analysis as an objective method to discriminate between genuine articles and forgeries, as well as for the classification of trace evidence such as accelerants, drugs, inks, and automotive paint by type, manufacturer or country of origin [272, 274, 275, 278, 279, 281, 283-285].

A similar approach might be used to infer the traits of an individual based on the composition of a fingermark, such as age or sex, if sufficient inherent diversity can be

identified between such groups. A similar approach has been used by Croxton *et al*. to highlight the compositional differences between charged and uncharged fingermarks [15].

5.1.4 Aims

This chapter describes the development and application of a gas chromatography-mass spectrometry method to the separation and identification of latent fingermark lipids from samples collected from a statistically relevant donor population. Compounds of interest were identified from the most abundant peaks commonly encountered in most samples, in conjunction with major sebum and fingermark constituents described in the literature. Principal component analysis was performed on this data to assess the effects of intra- and inter-donor variation on fingermark composition.

5.2 Experimental

5.2.1 Chemicals

Myristic acid (≥99.5 %; Aldrich, USA), palmitic acid (≥99 %; Fluka Analytical), sapienic acid (99 %; Matreya, USA), palmitoleic acid (≥98.5 %; Sigma-Aldrich, USA), stearic acid (≥99 %; Aldrich, USA), squalene (≥98 %; Sigma-Aldrich, USA), cholesterol (≥99 %; BDH, UK), myristyl palmitoleate (99 %; Nu-Chek Prep, Inc, USA), myristyl palmitate (99 %; Nu-Chek Prep, Inc, USA), palmityl palmitate (99 %; Nu-Chek Prep, Inc, USA), palmityl palmitoleate (99 %; Nu-Chek Prep, Inc, USA), oleyl myristate (99 %; Nu-Chek Prep, Inc, USA), USA), stearyl myristate (99 %; Nu-Chek Prep, Inc, USA), stearyl palmitoleate (99 %; Nu-Chek Prep, Inc, USA), palmityl oleate (99 %; Nu-Chek Prep, Inc, USA), stearyl palmitate (99 %; Nu-Chek Prep, Inc, USA) and dichloromethane (≥99.9 %; 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.

5.2.2 Sample collection and storage

Samples were collected from 10 donors for method development, while samples from up to 116 donors were collected for the investigations. Fingermark samples were collected on filter paper circles (25 mm qualitative filter paper, Grade 1; Whatman, UK). Additional samples collected for method optimisation were collected on A4 white copy paper (80 g/m^2; Fuji Xerox Professional), quartered glass microfibre filters (47 mm GF/C grade; Whatman, UK) and glass microscope slides (Esco Optics, USA) that had been cleaned with

methanol, deionised water and detergent, and annealed before use. Prior to sample collection, donors were asked to read and sign a consent form (Appendix 1). 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 fingermarks from both hands were deposited sequentially to collect two fingermarks on each filter paper. After the donor removed their hand, the filter papers were wrapped in aluminium foil and labelled with an alphanumeric code (described in Chapter 2). Donors were also asked to fill out a brief survey (Appendix 1), regarding their age, sex and recently handled substances. 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 outside of Curtin University's Bentley campus were stored in an ice box until they had been transported to either the laboratory or the freezer.

5.2.3 Sample preparation

Sample preparation was based on the methodology described by Koenig *et al*. [14]. Extraction of fingermark 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 extraction from the rubber septa, and analysed by GC-MS. Analytical blanks consisting of clean filter papers were prepared and analysed with each set of samples.

5.2.4 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. The number of instruments

used is reflective of changing instrument availability. Full instrumental conditions are described in Table 5.1.

Table 5.1: Instrumental conditions for GC-MS

	Gas chromatograph	Column type	Injector	Injection volume	Mass spectrometer
Method development	Hewlett Packard 6890 series	Agilent J&W DB-5MS (60 m x 0.25mm ID x 0.25 µm d_f)	Hewlett Packard 6890 series injector	1 µL	Hewlett Packard 5973 MSD
	6890N series	Phenomenex ZB-5MS (30 m x 0.25 mm ID x 1 µm d_f)	Gerstel MPS2 autosampler		Agilent 5973N
C16:1 isomer comparison	Agilent 7890A	Agilent Technologies HP-Innowax (30 m x 0.25 mm ID x 0.25 µm d_f)	Agilent 7683B series		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 µm 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 µm 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 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.

5.2.5 Data analysis

The data was pre-processed using Chemstation Data Analysis (Agilent Technologies, USA) by background subtraction of all chromatograms, followed by manual integration of selected peaks (see section 5.3.1.2). 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).

5.3 Results and discussion

5.3.1 Method development

5.3.1.1 Sample collection and preparation

An ideal substrate for chromatographic analysis of latent fingermarks would enable the collection and extraction of analysable quantities of lipid material, and introduce minimal quantities of contaminants into the extract. Additionally, the substrate ought to be representative of those commonly encountered in forensic investigations. Procedures for sample collection vary considerably between GC-MS studies of fingermarks, making it difficult to compare quantitative results [15]. Some researchers have opted to deposit (usually charged) latent fingermarks onto substrates such as glass fibre filter papers [10-12, 14], Mylar film [15, 32], or glass [14, 41, 256]. Fingermarks deposited on porous substrates typically contain more total material than those deposited on nonporous surfaces, due to absorption into the substrate [13, 120]. The porous substrates mentioned above are very different to common paper substrates, and may not interact with fingermark residue during and after deposition in the same manner.

Preliminary investigations were carried out to ascertain the best substrate for sample collection for subsequent analysis, using a range of porous and nonporous substrates commonly utilised in the literature, as well as several alternatives. It was found that greater peak signals were obtained in chromatograms of samples extracted from porous substrates, compared to extracts from glass microscope slides (Figure 5.1).

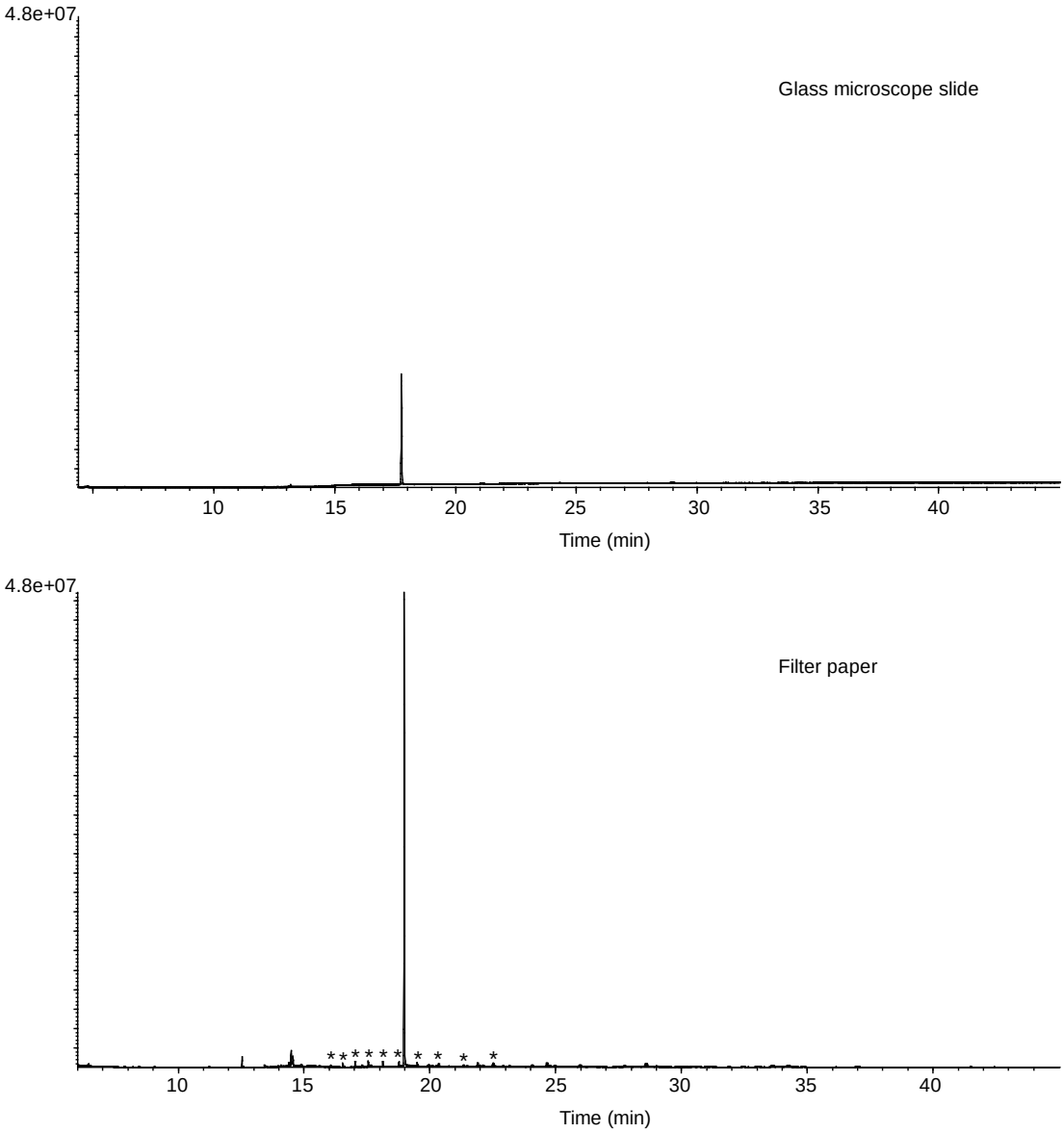


Figure 5.1: Effect of substrate porosity on total ion chromatogram (TIC) signal. Peaks introduced from substrate are indicated with asterisks. Disparities in retention times are due to different instruments used.

Both sample extracts and blanks from white copy paper, filter paper and glass microfibre filters contained a number of contaminants, identified using the MS library, in conjunction with visual examination of the mass spectra, as long-chain alkanes. As these peaks were not observed in blank or sample extracts from microscope slides, they were presumed to originate from the porous substrates. The presence of contaminants in fingermark samples derived from extraction from porous substrates has been previously reported [14]. Though their relative retention times appear similar to the long-chain alkanes observed in this study, the identities of these compounds were not reported by Koenig *et al*. It has been

demonstrated that a number of organic contaminants, including long-chain alkanes, may be derived from paper-based sources [286]. 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 [7]. Attempts to pre-clean porous substrates by sonication for 15 minutes in dichloromethane were unsuccessful in completely removing these compounds.

Comparisons of blanks and fingermark extracts from glass fibre filters, filter paper and white copy paper found that glass fibre filters produced the lowest signals for alkane peaks, but also produced low sample peak signals. Filter paper and copy paper were found to introduce similar peak signals from the alkane contaminants. Based on these observations, filter paper circles were used for sample collection in all subsequent investigations, though it is recognised that filter paper cannot be considered entirely representative of commonly encountered porous substrate types [120]. Additionally, the size of the filter paper circles (2.5 cm diameter) provided a convenient means to collect and extract samples compared to A4 sheets of copy paper.

As discussed in Chapters 2 and 4, the use of charged fingermarks in fingermark detection research is a contentious issue. While natural or uncharged latent fingermarks may still contain enough lipid material to enable their detection on paper substrates, to enable comparisons with the results of published studies [10, 11, 14], charged fingermarks were used in the analytical investigations carried out in this thesis. It should be noted that results by Croxton *et al*. indicate that the common practice of charging fingermarks in analytical and development reagent studies might cause sebaceous compounds to be overrepresented, such that charged and uncharged fingermarks may be clearly resolved on a PCA scores plot [15]. They concluded that charging fingermarks may therefore not be representative of actual latent fingermarks left at crime scenes, and that analysis of uncharged fingermarks should be performed at least alongside charged samples.

The primary aim of this study is to investigate compositional variation within a larger donor population than in previous studies described in the literature, in order to produce statistically valid data. This required the collection of hundreds of fingermark samples. To facilitate the GC-MS analysis of such a large number of samples and reduce sample preparation times, a simple extraction method was therefore desired. Sample preparation was based on the methods described by Asano *et al*., Koenig *et al*. and Weyermann *et al*. [13, 14, 41], to enable the results obtained in this study to be more comparable to these

previous studies. These approaches enable the detection of up to almost 100 lipid compounds, representing most major lipid classes present in sebum [12], while avoiding time-consuming derivatisation procedures [120]. Due to the small amount of material that comprises latent fingermarks, 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 [10, 13, 14, 41, 44]. Such a step was not employed in the method used in this thesis 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.

5.3.1.2 Analytical conditions

The temperature program used was selected due to its similarity to the analytical conditions used in previous studies [13, 14], and was found to enable separation of saturated and monounsaturated free fatty acids, squalene, cholesterol and wax esters. The only major class of fingermark lipids not detected with this method was triglycerides. GC-MS of intact triglycerides requires oven temperatures beyond the limits of the columns used in this study, and so this was not investigated any further [287, 288].

5.3.1.2 Data analysis

A relative quantification approach was chosen over absolute quantification to overcome variation in the amount of residue deposited by donors due to differences in fingermark size or deposition technique. There is no correlation regarding the contribution of lipid material to total fingermark mass [132], and quantitative differences may be a factor of fingermark surface area rather than donor trait [13, 15]. 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 [29]. As found during the investigations conducted in Chapter 4, donors often do not reproducibly deposit impressions of the entire fingermark pattern (Figure 5.2), which may contribute to intra-donor variation.

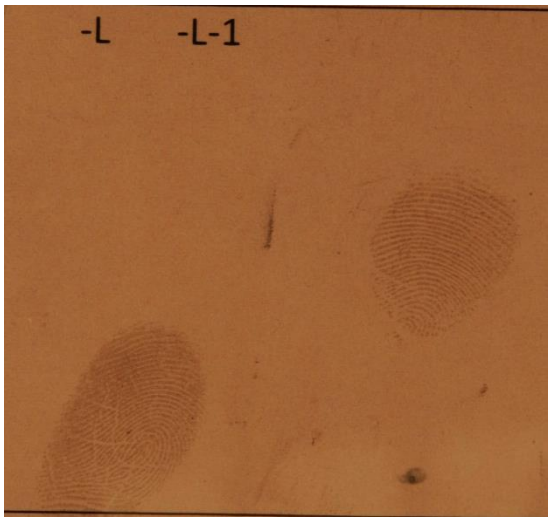


Figure 5.2: Sample image from the donor study (Chapter 4) treated with Oil red O and physical developer, showing uneven deposition of ridge patterns in two fingermarks deposited simultaneously from the same hand

Fifteen components including free fatty acids, squalene, cholesterol and some wax esters were identified as the abundant peaks common to most fingermark samples (further details provided below in section 5.3.2.1). The peak areas of each compound were normalised against the sum to determine the relative quantities of each compound in the samples. Some latent fingermark studies have used internal standards to observe relative changes in composition with time [10, 14, 44] or have determined composition based on peak area ratios to squalene [41]. Normalisation to the sum has been demonstrated to reduce intra- and inter-sample variability compared to normalising to an internal standard [12]. Normalisation to the sum was compared to normalisation against the square root of the sum of squares, which can further reduce the influence of this variability on a PCA model. The resultant scores plots showed no significant differences in projection. As such, the former normalisation method was used with all datasets.

5.3.2 Compound identification

5.3.2.1 Endogenous lipids

A range of endogenous lipid compounds reported in previous fingermark studies [10, 11, 13-15, 41] were identified in fingermark samples in these investigations. A sample chromatogram of a latent fingermark is shown in Figure 5.3. 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 (Table 5.2). 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 [11, 13, 14, 44, 132].

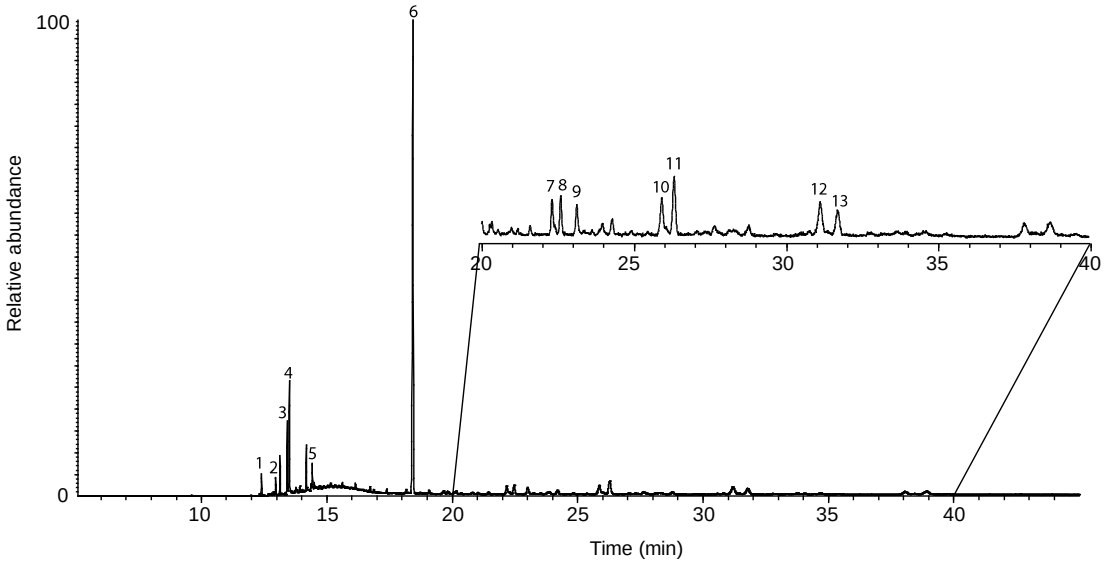


Figure 5.3: Sample total ion chromatogram (TIC) of a latent fingermark, showing identified peaks 1. Myristic acid, 2. Pentadecanoic acid, 3. Hexadecenoic acid, 4. Palmitic acid, 5. Oleic acid, 6. Squalene, 7. C30:1 wax esters, 8. C30:0 wax esters, 9. Cholesterol, 10. C32:1 wax esters, 11. C32:0 wax esters, 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 [84], 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 [49, 84, 86, 87]. The co-elution of isomeric wax esters was confirmed from a combined examination of the mass spectral data and retention time comparisons with standard solutions.

Table 5.2: Compounds identified in chromatograms of fingermark 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)	MS library comparison, examination of MS
Lauryl palmitate (12:0-16:0)	Examination of MS
Stearyl decanoate (18:0-10:0)	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)	MS library comparison, examination of MS, standard
Myristyl palmitate (14:0-16:0)	Examination of MS, standard
Stearyl laurate (18:0-12:0)	Examination of MS
Lauryl stearate (12:0-18:0)	Examination of MS
Decyl eicosanoate	Examination of MS
Cholesterol	MS library comparison, standard
Wax esters (C32:1)	
Palmityl hexadecenoate (16:0-16:1)	Examination of MS
Myristyl oleate (14:0-18:1)	Examination of MS
Oleyl myristate (18:1-14:0)	Standard
Wax esters (C32:0)	
Palmityl palmitate (16:0-16:0)	MS library comparison, examination of MS, standard
Stearyl myristate (18:0-14:0)	Examination of MS, standard
Myristyl stearate (14:0-18:0)	MS library comparison
Lauryl eicosanoate (12:0-20:0)	Examination of MS
Wax esters (C34:1)	
Stearyl hexadecenoate (18:0-16:1)	Examination of MS
Palmityl oleate (16:0-18:1)	Examination of MS, standard
Wax esters (C34:0)	
Stearyl palmitate (18:0-16:0)	MS library comparison, examination of MS, standard
Palmityl stearate (16:0-18:0)	Examination of MS
Arachidyl myristate (20:0-14:0)	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 (Table 5.3) [84, 85, 87, 289]. 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.

Table 5.3: Diagnostic fragment ions used to identify fatty acids (R) and fatty alcohols (R') of wax esters

Saturated fatty acids			**Monounsaturated fatty acids**		
	$[RCO_2H_2]^+$	**$[RCO]^+$**		**$[RCO_2H_2]^+$**	**$[RCO]^+$**
Decanoic acid	173	155	Decenoic acid	171	152
Dodecanoic acid	201	183	Dodecenoic acid	199	180
Tetradecanoic acid	229	211	Tetradecenoic acid	227	208
Pentadecanoic acid	243	225	Pentadecenoic acid	241	222
Hexadecanoic acid	257	239	Hexadecenoic acid	255	236
Octadecanoic acid	285	267	Octadecenoic acid	283	264
Eicosanoic acid	313	295	Eicosenoic acid	311	292
Saturated fatty alcohols			**Monounsaturated fatty alcohols**		
	$[R'CO_2]^+$	**$[R'-H]^+$**		**$[R'CO_2]^+$**	**$[R'-H]^+$**
Decanol	185	140	Decenol	183	138
Dodecanol	213	168	Dodecenol	211	166
Tetradecanol	241	196	Tetradecenol	239	194
Pentadecanol	255	210	Pentadecenol	253	208
Hexadecanol	269	224	Hexadecenol	267	222
Octadecanol	297	252	Octadecenol	295	250
Eicosanol	325	280	Eicosenol	323	278

Some wax ester standards were found to have a slightly longer retention time than the fingermark 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 elute 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 [84, 86], 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 [84]. 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 [265]. Another possibility is that the monounsaturated wax esters are positional isomers of the standards, which can affect retention times [290, 291], 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 fingermarks.

A characteristic feature of human sebum is the prevalence of the Δ6 pattern of unsaturation [40, 47, 70, 82, 87, 260]. The majority of unsaturated free fatty acids, wax esters and sterol esters produced by adult human sebaceous glands display an unusual desaturation position of Δ6 rather than the more typical Δ9 pattern [39, 40, 70, 79]. No studies of fingermark composition have reported the detection of sapienic acid (C16:1Δ6), while its isomer palmitoleic acid (C16:1Δ9) is identified as one of the most abundant monounsaturated fatty acids in fingermark residue [10, 11, 41, 120]. Several studies have reported the detection of wax esters containing palmitoleic acid or palmitoleyl alcohol in latent fingermarks [14, 25, 41]. Conversely, in dermatological research, while sapienic acid is often mentioned as a major component of sebaceous free fatty acids, palmitoleic acid is not [87]. Work by Pappas *et al.* found that exogenously applied, 3H labelled palmitoleic acid was not incorporated into wax esters except as extension products [58].

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 [14, 15, 50]. 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 [291]. As the identity of the monounsaturated C16 fatty acid could not be confirmed, it is referred to throughout this thesis 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 [12, 14]. 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 classification 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 by the greater inter-donor discrimination that would be possible compared to only utilising those common to samples from all donors [12].

5.3.2.2 Exogenous contaminants

In this study, donors were not asked to wash their hands prior to sample collection 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 there was interest in determining if the use of skin products could be demonstrated using PCA. Free fatty acids, squalene, cholesterol and wax esters are all common ingredients of skin products [10, 85, 86], and therefore the presence of such in fingermark samples may have an effect on classification compared to samples consisting only of skin secretions.

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 5.4). 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 [292]. 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 [292], and was frequently encountered in the fingermarks of donors who used such products. Additional peaks, identified as long-chain alkanes, were determined to have been extracted from the filter paper, as discussed in section 5.3.1.

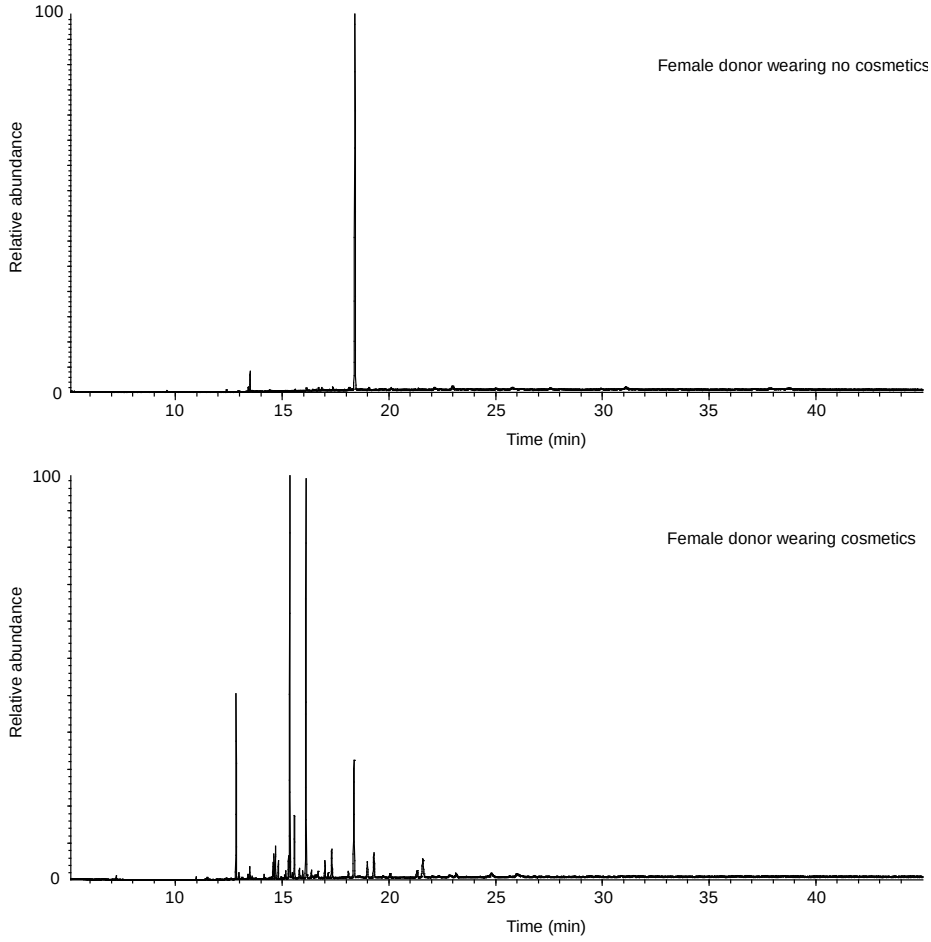


Figure 5.4: Example of exogenous peaks introduced into TICs of samples from two adult female donors by use of cosmetic products

5.3.3 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 [10, 12-14, 41]. If an individual's fingermark composition was shown to vary significantly over time, and this variance as great, or greater, than that observed between different individuals, using fingermark composition as a means to infer individual characteristics or estimate fingermark age could not be considered a viable approach [133].

5.3.3.1 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 [38, 66]. While the secretion rate of the sebaceous glands of the forehead demonstrates a circadian rhythm, with maximal rates around midday, to the best of this author's knowledge, it is not known if this is reflected in the relative concentrations of individual constituents [110-112]. To investigate whether fingermark lipid composition was affected by time of day, samples were collected in triplicate from five donors (Table 5.4) 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 greasy substances.

Table 5.4: Summary of donor information

Sex	***n***	**Age (years)**	***n***	**Recent skin product use**	***n***
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 (Figure 5.5). The Scree plot was used as an evaluation tool to determine the number of PCs that may be used to model the data, based on the levelling-off of the plot [275, 276, 293]. Utilisation of all PCs may introduce noise from spectral or chromatographic data into the model, whereas too few may result in the omission of meaningful information from within the original dataset [271, 276, 293]. In the Scree plot below, it can be seen that the first two PCs may be sufficient to model the data; however, the inclusion of the third PC into the model may highlight subtle differences between data points and enable their separation on a scores plot [279].

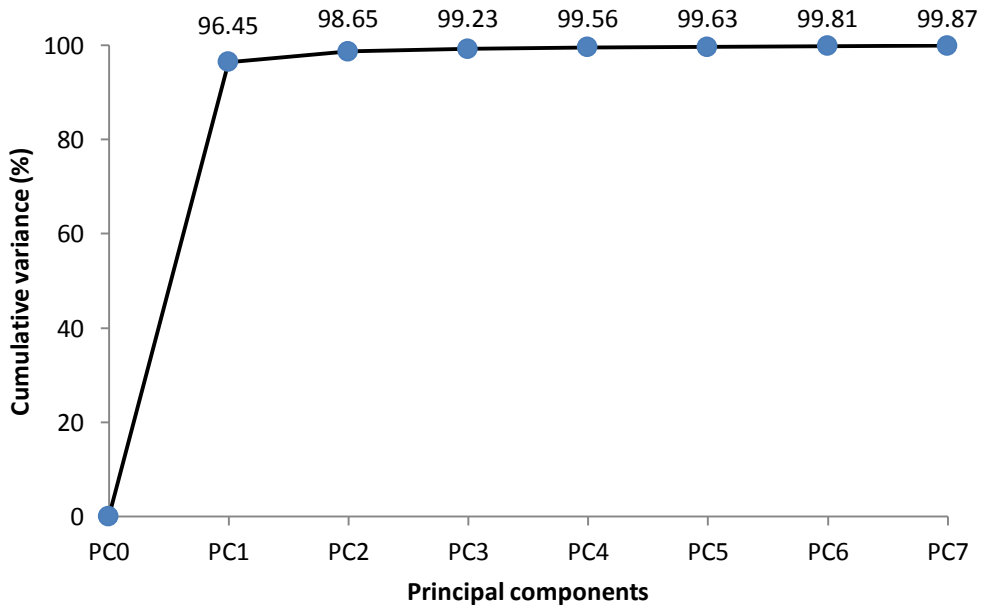


Figure 5.5: Scree plot depicting the variance in the dataset accounted for by each PC

The scores plot generated from the first 3 PCs (Figure 5.6) 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.

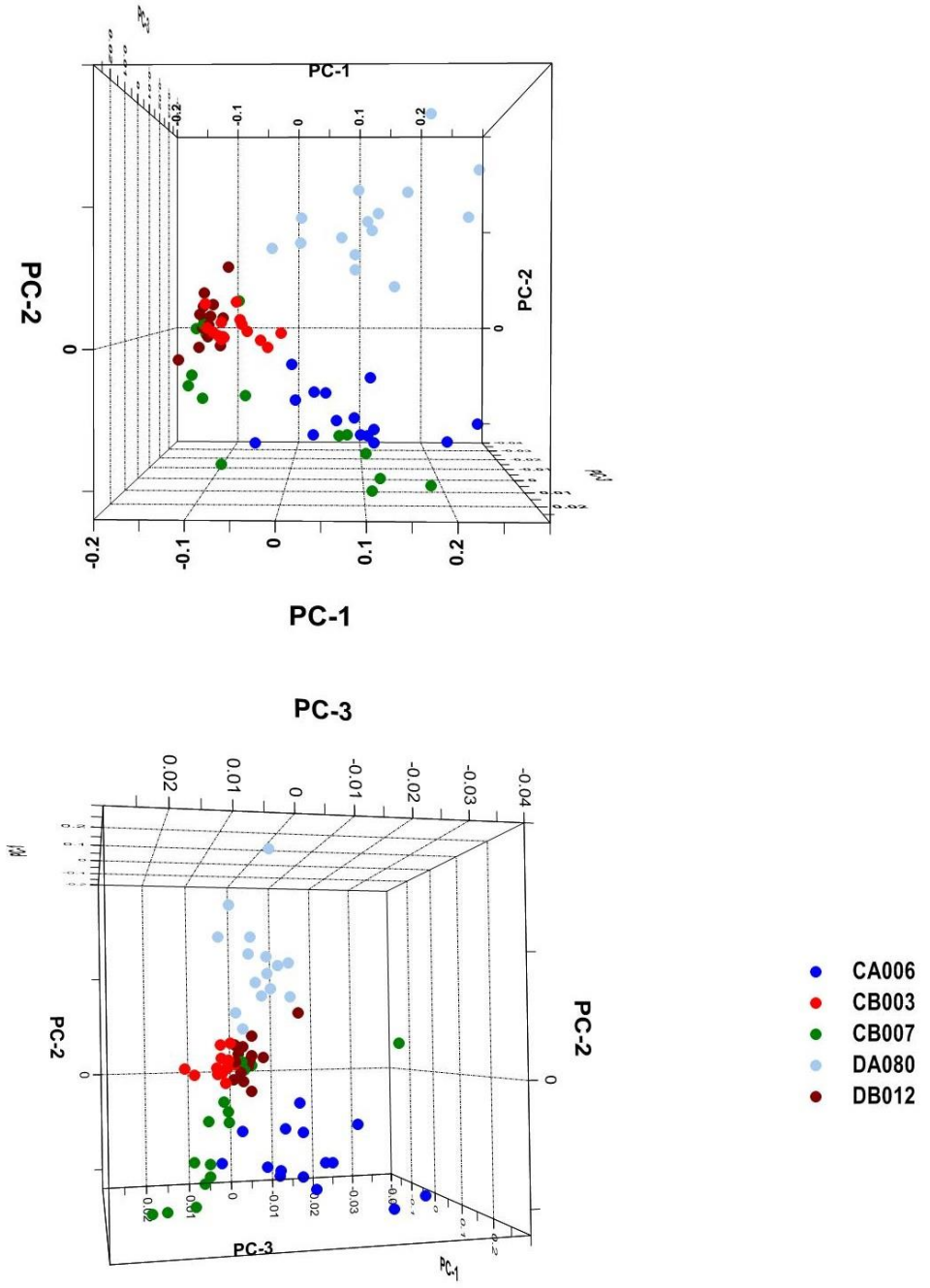


Figure 5.6: 3-dimensional scores plot generated from the first 3 PCs, from two perspectives, demonstrating the distribution of fingermarks collected from five donors over the course of eight hours

For most donors, replicate samples from the same sampling time were scattered throughout each cluster, indicating that there was no clear trend in fingermark 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 fingermarks 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 5.7). 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 fingermarks. 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 [49, 76]. 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 [7, 14, 64, 66, 67]. Discrimination between samples therefore arises primarily from differences in relative amounts of the most abundant lipid compounds – squalene and long-chain free fatty acids [10, 11, 14].

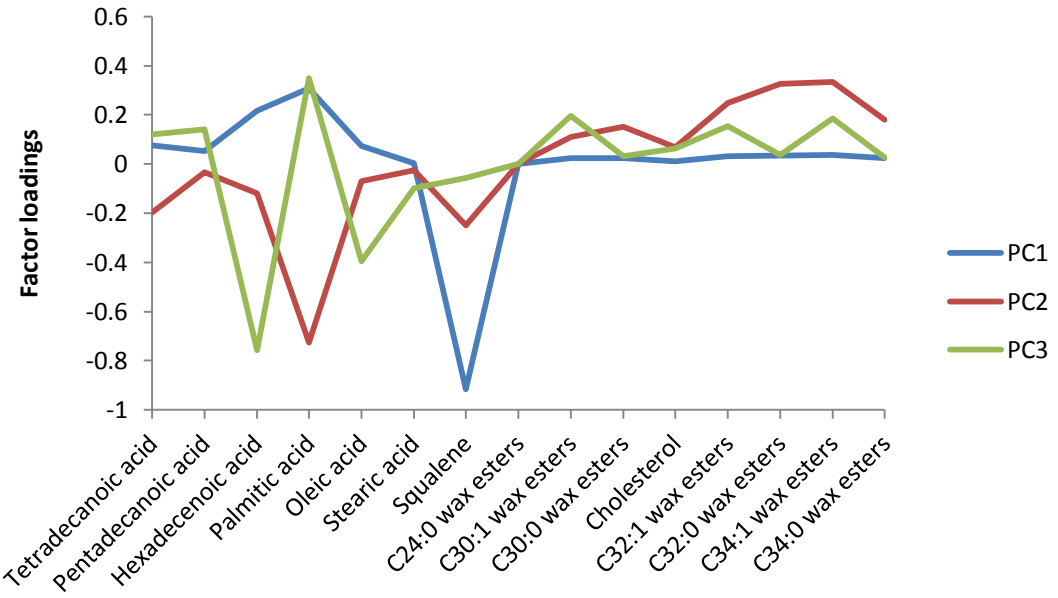


Figure 5.7: Factor loadings for the first 3 PCs

Several investigations have concluded that although there was some variation in the concentrations of some fingermark lipids over the course of a day, this variation was not statistically significant [10, 41, 256]. Koenig *et al*. observed large variation similar to that of Archer *et al*. and cited this as an issue in sample reproducibility, but did not determine the statistical significance of this variation [14]. 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 [10, 13, 14, 256]. The study reported here shows that monitoring intra-donor changes in one donor 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 fingermark development methods utilise fingermarks from at least 3 – 5 donors [191]. This approach may benefit analytical studies of fingermark composition in demonstrating compositional variation.

5.3.3.2 Variation over one month

Similar to the studies discussed in section 5.3.3.1, previous reports of intra-donor variation of fingermark composition over periods of several days to weeks have typically monitored one donor, or have only sampled at infrequent intervals [11, 13, 14]. 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 that 99.26 % of the total variance of the dataset was accounted for in the first 3 PCs, as depicted in the Scree plot (Figure 5.8). The scores plot generated using the first 3 PCs (Figure 5.9) was largely similar in appearance to Figure 5.6 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.

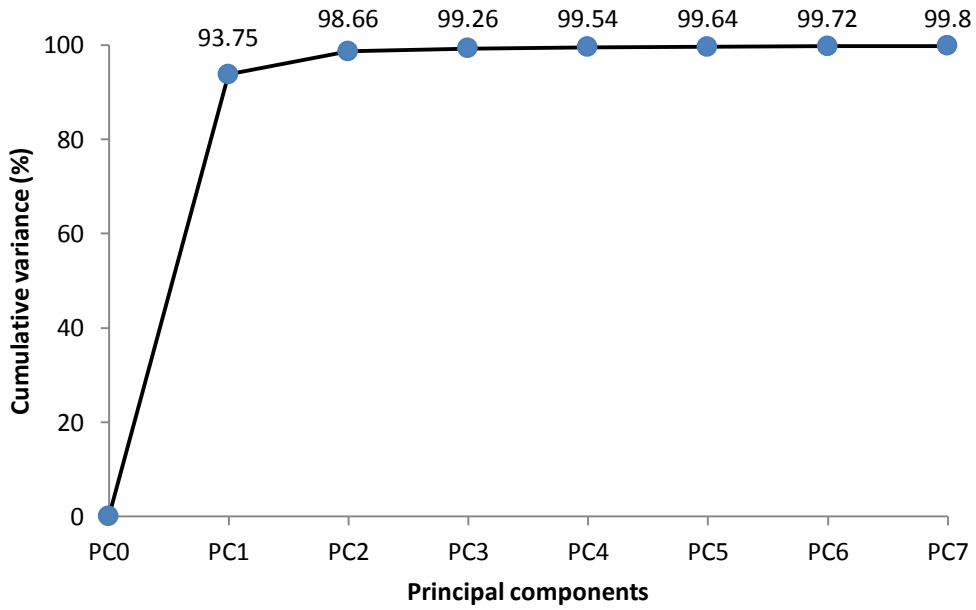


Figure 5.8: Scree plot depicting the variance in the dataset accounted for by each PC

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 [10, 11, 14]. 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 [14, 294], 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.

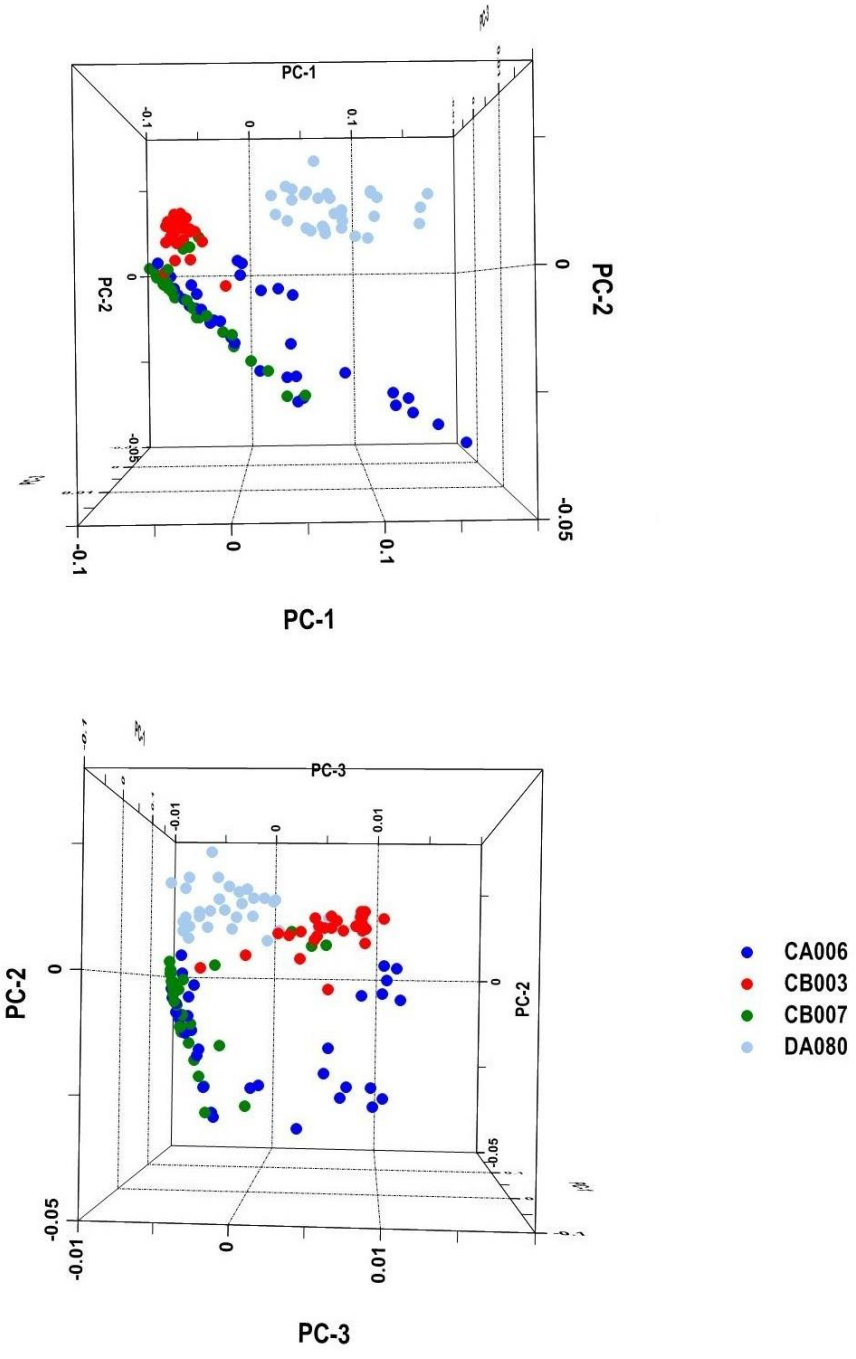


Figure 5.9: 3-dimensional scores plot generated from the first 3 PCs, from two perspectives, demonstrating the distribution of fingermarks collected from four donors over 29 days

The factor loadings for the first 3 PCs were utilised to identify the compounds that contributed to the variance within the dataset (Figure 5.10). The loadings plots for the first

two PCS were almost identical to those in Figure 5.7. The loadings plot PC1 revealed significant negative correlation to squalene, as well as some positive correlation to palmitic acid and hexadecenoic acid. The loadings plot for PC2 revealed significant negative correlation to palmitic acid, and some positive correlation to several wax esters. The loadings plot 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 as in section 5.3.3.1, 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.

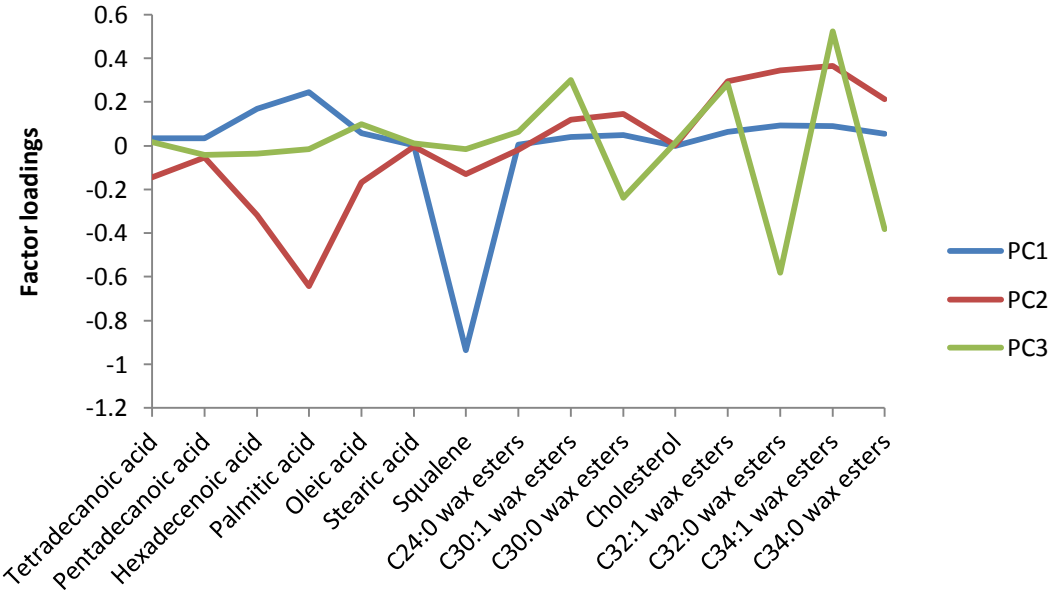


Figure 5.10: Factor loadings for the first 3 PCs

It has been suggested that the use of skin products may affect the consistency of latent fingermark 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 (detailed in section 5.3.4 and Chapter 6) were found to differ noticeably in appearance (Figure 5.11). 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 fingermark composition, possibly 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 [52].

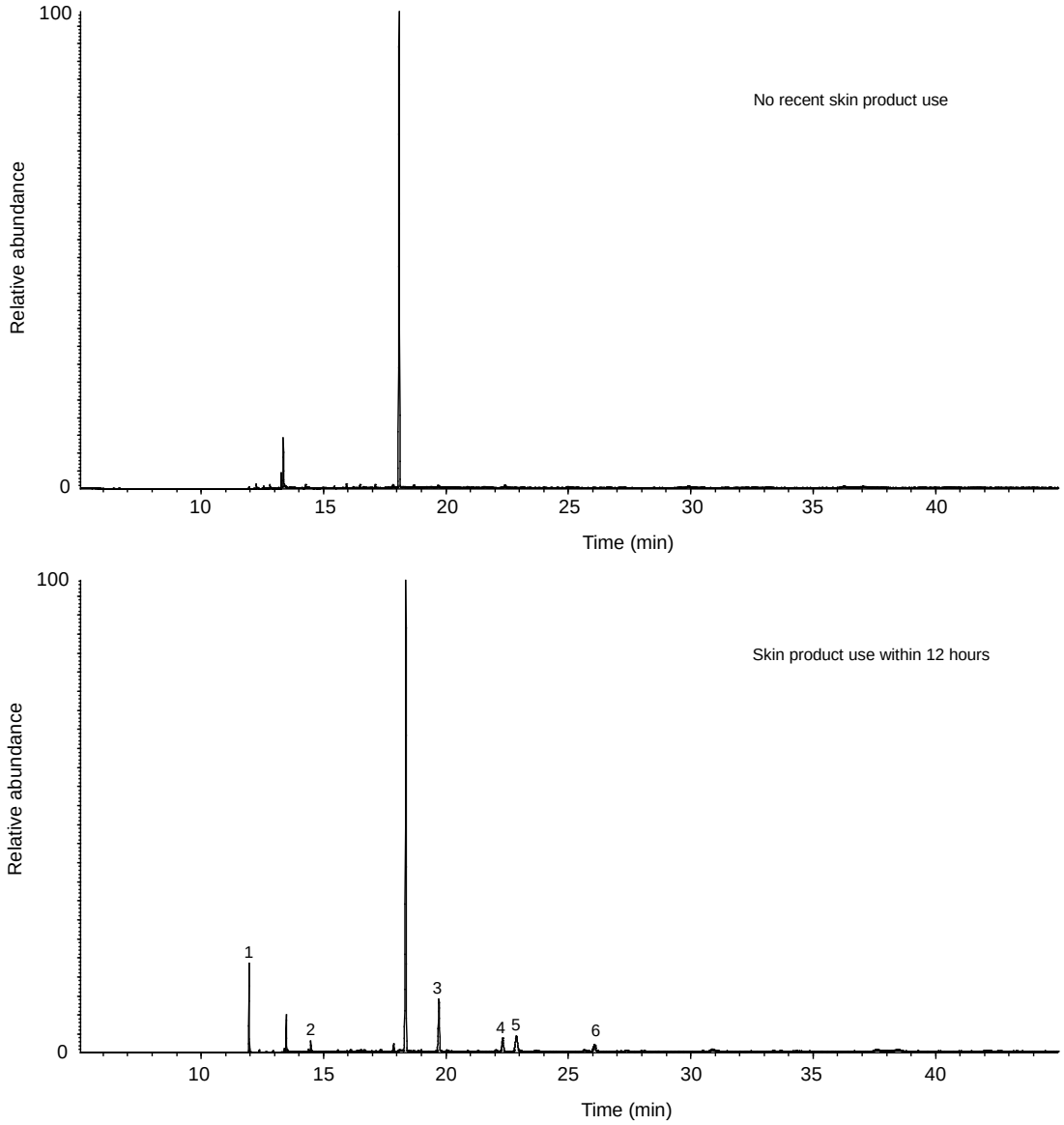


Figure 5.11: TICs of fingermark samples collected from an adult male donor showing identified peaks related to use of skin products 1. Unknown compound, 2. Stearic acid, 3. Myristyl myristate, 4. Myristyl palmitate, 5. Vitamin E acetate, 6. Myristyl stearate

5.3.4 Inter-donor variation

The results of the above investigations indicate that differences in initial fingermark lipid composition are greatly influenced by inter-donor variation. This investigation aimed to determine if significant differences could be observed in the composition of fingermarks 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 6 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 5.5. 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 5.5: 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**

The total number of donors and wide age range was chosen in order to provide a more representative subset of a population than has been achieved in previously reported investigations (Table 5.6). Many studies into fingermark composition using GC-MS have utilised rather small numbers of adult donors, presumably individuals immediately available within the laboratory, and as such are rather limited in providing statistically valid information regarding inter-donor variation [10, 14, 15, 256].

Table 5.6: Comparison between donor population of this study and those of previous GC-MS studies of latent fingermark composition

	Total (*n*)	**Male (*n*)**	**Female (*n*)**	**Adult (*n*)**	**Child (*n*)**
Donor population	**116**	**59**	**57**	**74**	**42**
Buchanan *et al*. [44]	*ca.* 50	n/a	n/a	n/a	n/a
Mong *et al*. [11]	79	40	39	47	32
Asano *et al*. [41]	20	10	10	n/a	n/a
Archer *et al*. [10]	5	5	0	5	0
Croxton *et al*. [15]	18	9	9	18	0
Weyermann *et al*. [13]	6	3	3	6	0
Koenig *et al*. [14]	6	2	4	6	0
Michalski *et al.* [256]	37	22	15	37	0
Girod *et al*. [12]	25	12	13	25	0

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 several 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' [11, 44], 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 5.12), while no fingermark material at all was detected in many others. As resampling from donors who only afforded blank chromatograms was not practical, the data from 33 donors (including all donors under the age of 10) were not included in the PCA model.

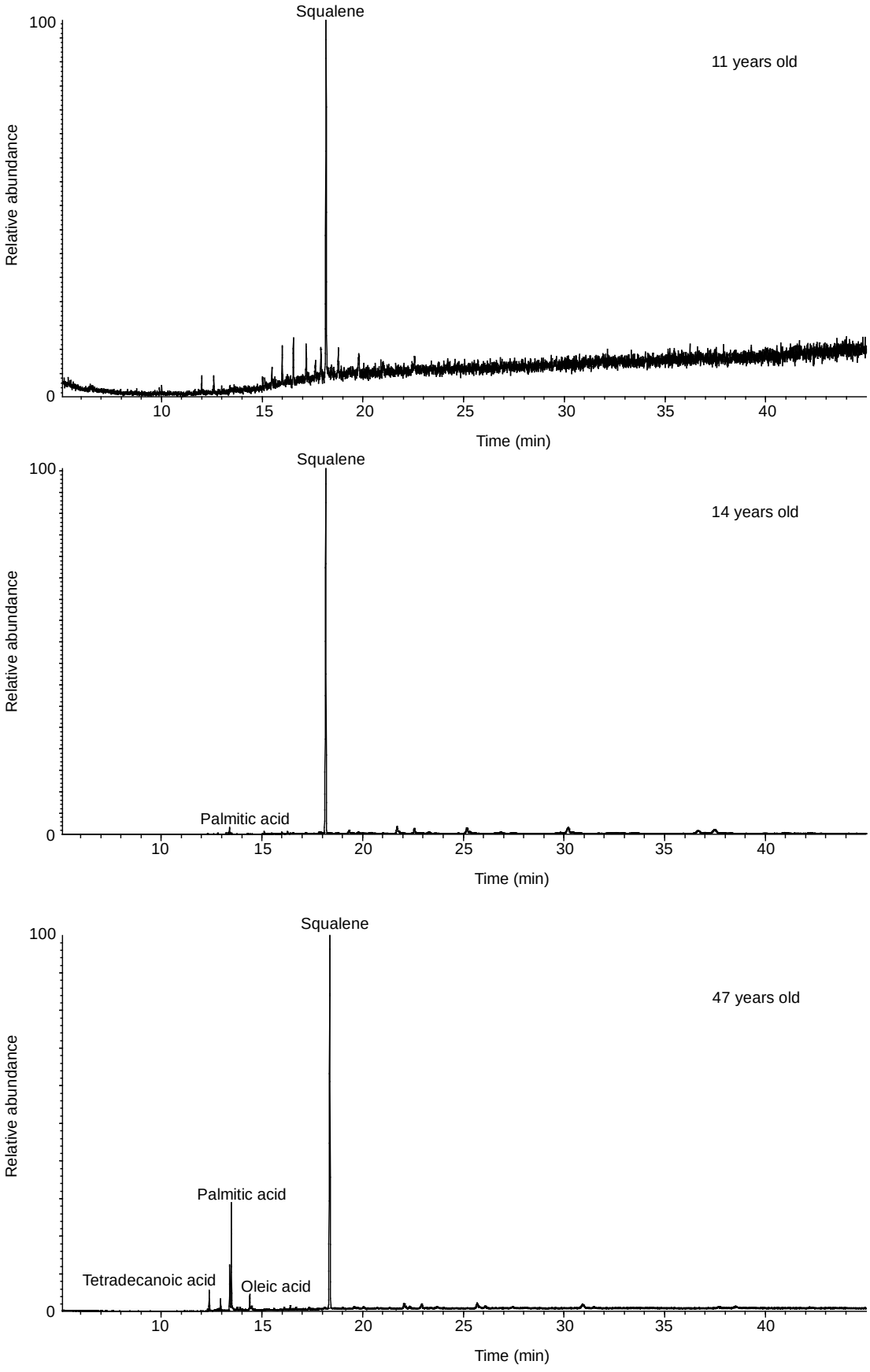


Figure 5.12: Comparison of TICs of samples collected from female donors of various ages

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, this is not a practical approach in an operational context, where only part of a single fingermark may be available [127]. 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 [12-14, 41]. Such an approach may be beneficial in the analysis of fingermarks of young children and other weak donors, however as discussed in section 5.3.1.1, 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 fingerprints, which typically do not contain significant amounts of sebaceous lipids [98, 103, 295].

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 5.13). Examination of the Scree plot showed that as many as 5 PCs could be utilised to adequately model the data. 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 previously in this chapter.

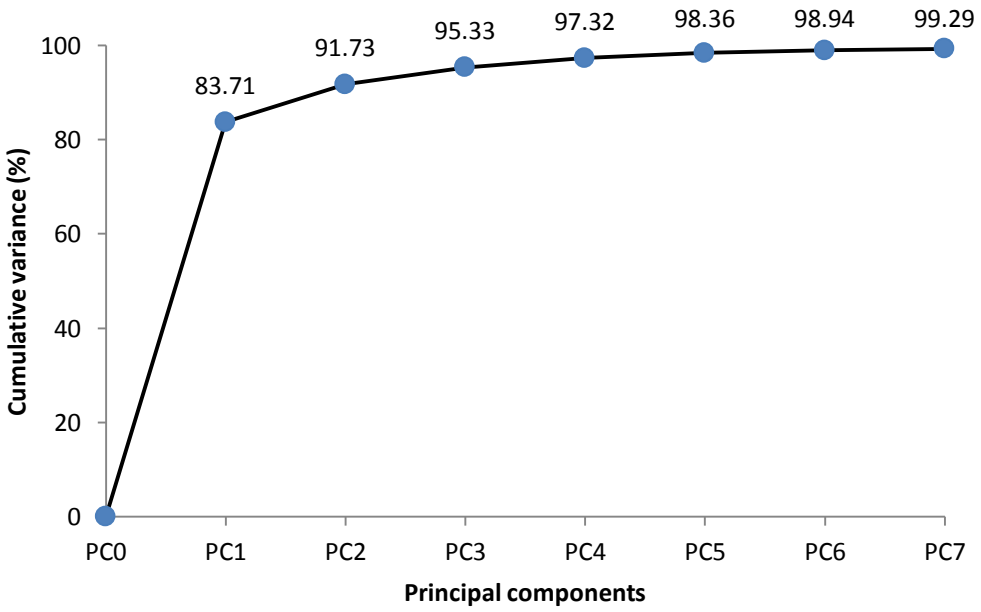


Figure 5.13: Scree plot depicting the variance in the dataset accounted for by each PC

Examination of the scores plot constructed using the first 3 PCs (Figure 5.14) 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 [15]. 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 [13, 15, 256]. 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 [11, 15, 44], it was thought that discrimination between individual donors might be possible. However, the use of a large sample size 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 5.15). The loadings plot for PC1 was again almost identical to those discussed in section 5.3.3, 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 [64]. 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 plot for PC2 revealed significant positive correlation to stearic acid, as well as significant negative correlation to palmitic acid and some negative correlation to squalene. The loadings plot for PC3 revealed significant positive correlation to hexadecenoic acid, and some negative correlation to palmitic and stearic acid. The differences in factor loadings for the second and third PCs may be attributed to the greater diversity of the sample population in this investigation, which is more representative of a general population than the smaller populations sampled from in section 5.3.3. 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.

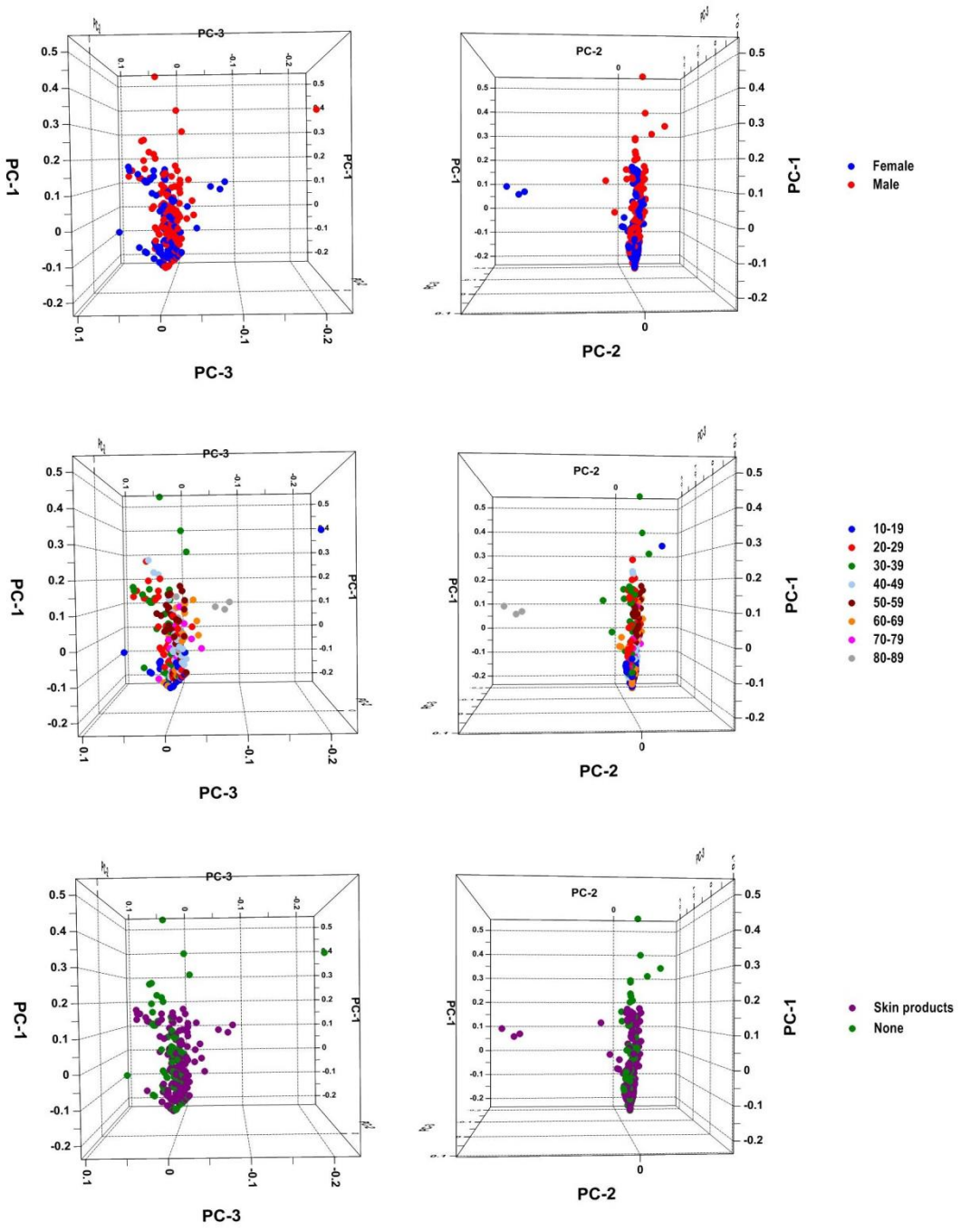


Figure 5.14: 3-dimensional scores plot generated from the first 3 PCs, demonstrating the distribution of fingermarks collected from 83 donors. Samples are colourised by biological sex (top), donor age in decades (middle), and recent use of skin products (bottom)

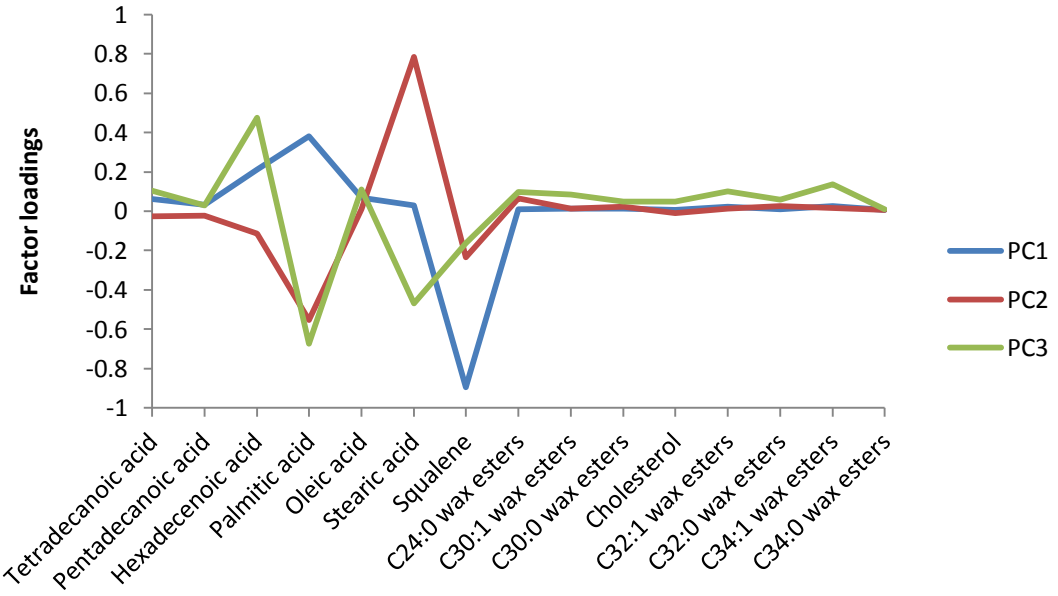


Figure 5.15: Factor loadings plots for the first 3 PCs

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 fingermark residue was deposited, including angle of contact (i.e. depositing material from the ends of the fingertip rather than the whole fingermark), time spent charging fingermarks, size of donors' fingermarks, pressure of application. These and other factors are thought to contribute to fingermark composition, though the means of such are not completely understood. It may be that some donors charged or deposited their fingermarks unequally by applying dissimilar pressure between the three middle fingers [10], or that there was variation in the skin lipid composition across the donor's forehead. These factors may account in part for the observation that fingermark composition varies with digit and handedness [119, 120].

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 [64]. Measuring the relative amounts of triglycerides and free fatty acids may therefore enable greater discrimination at an individual level, if correlations to age, sex or other traits are not possible. To do so would require modification of the methodology presented in this thesis, which is not amenable to the separation of triglycerides due the maximum temperature

limits of the columns available. Liquid chromatography may be a more suitable method for separation of triglycerides, though GC-MS methods for the simultaneous separation and detection of all sebaceous lipid classes have been reported [49]. In any case, the characterisation of sebum triglycerides is complicated by the current lack of knowledge regarding their structure [4, 49], and it is expected that the mass spectra of such would be complicated by the co-elution of isomers, in much the same manner as the wax esters [49].

Penn *et al*. suggested that the profiling of entire chromatograms, rather than selected compounds, may achieve individual classification of volatile skin compounds [269]. While this may not be practical with latent fingermarks, 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 [12]. 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 contamination.

5.4 Conclusion

A simple extraction followed by analysis with gas chromatography-mass spectrometry was successful in confirming the presence of a number of sebaceous components in latent fingermarks collected on porous substrates. The inherent difficulties in obtaining reproducible fingermark samples were recognised as possible functions of sample deposition as well as compositional variation. Significant intra-donor variation was demonstrated to affect sample projection on PCA scores plots, though no trends related to this variation over short or long periods of time were observed.

The relative amounts of squalene, hexadecenoic acid and palmitic acid appeared to account for the majority of the variance within all datasets. While inter-donor variation in relative lipid abundances was observed, these differences were not sufficient to enable visual discrimination within a large donor population. Samples were projected too close together on a PCA scores plot to distinguish between individual donors, or traits such as age or biological sex.

The results discussed in this chapter emphasise the complexity of latent fingermark composition, and the challenges posed to current lines of research. The interplay of donor traits and deposition factors as influences on fingermark composition may never be completely understood due to their overlapping effects.

Chapter 6: Analysis of changes in fingermark lipid composition as a function of time by gas chromatography-mass spectrometry (GC-MS)

6.1 Introduction

The degradation processes that affect the properties of latent fingermarks can have a marked detrimental impact on the capabilities of forensic personnel to recover fingermarks within days of deposition [10, 11, 98, 102, 295, 296]. Conversely, there are several recorded instances of fingermarks several years old being detected against expectations [161, 297]. Furthermore, the degradation of fingermark components would undoubtedly impact upon any method to infer individual traits from fingermark composition [98]. Though there has been much call to address these issues, there is currently little detailed knowledge about the processes of fingermark degradation beyond broad trends.

In recent years, there have been several investigations into the changes in latent fingermark composition that occur as a function of time. The stated aims have included obtaining a better understanding of the process of fingermark degradation [10, 122], the identification of compounds which remain stable over time (or are stable degradation products) as potential targets for fingermark development [11, 122, 295, 298], as well as the development of a means to estimate the age of a fingermark for the purposes of criminal investigations [13, 14, 122, 124, 161, 297, 299].

6.1.1 Degradation of latent fingermark lipids

Latent fingermark composition begins to alter very soon after deposition, as evidenced by difficulties in developing fingermarks of increasing age with many detection methods [7, 10, 121, 122, 300]. Storage conditions, microbial activity, and the application of development reagents are all thought to impact upon the rate and types of changes that may occur [8, 10, 14, 122, 123, 297]. The initial composition of a latent fingermark also has great influence on its longevity [10, 13, 121]. Environmental factors, including light exposure, substrate type, temperature, humidity, airflow and immersion in water, are known to play a significant role in degradation rate; however, little is understood about their specific impacts upon fingermark chemistry [5, 13, 45, 103, 109, 121, 124, 194, 295, 297, 301].

The lipid fraction comprises the more durable portion of latent fingermark residue, due to its hydrophobic and non-volatile nature. It also highly subject to chemical modifications, and so it is this fraction of latent fingermarks which has been studied most extensively in regards to changes in the composition over time [10, 11, 13, 44, 98, 194, 300, 301]. Due to the inherent variability of fingermark samples, a timeframe of the degradation processes of the lipids has proved difficult to characterise in detail; so far only broad trends have been identified [10, 11].

An investigation into latent fingermark longevity by Buchanan *et al*. (detailed in Chapter 5) indicated that young children's and adults' fingermarks differed significantly in durability, due to quantitative differences in lipid content [44]. The first reported systematic analytical study that monitored fingermark degradation processes was conducted by Mong *et al*. in 1999, in recognition of the fact that latent fingermark detection is not often carried out on very recent deposits [302]. They also proposed that relatively stable degradation products might serve as target molecules for novel techniques geared towards the development of older fingermarks that existing methods are unable to detect. It was found that over a 60 day period, unsaturated compounds such as monoenoic fatty acids, wax esters, and squalene underwent oxidation to saturated compounds, or to shorter-chain, volatile degradation products.

The differences in longevity of children's and adults' fingermarks were further explored by Antoine *et al*., with the aim to determine how fingermark age impacted on the ability to estimate donor age using Fourier transform infrared (FTIR) microscopy [98]. It was found that latent fingermarks of prepubescent children and adults could be clearly distinguished even after 4 weeks following deposition, which was attributed to the abundances of cholesterol and sterol and wax esters, which is reflective of the differences in initial fingermark composition between the two age groups.

Archer *et al*. were the first to report the impact of storage conditions on degradation rate, by halving fingermark samples, and storing one half in either constant light or total darkness for a period of up to 33 days [10]. For the most part, the results obtained were consistent with the findings of Mong *et al*., but additionally, fingermark degradation was found to occur more rapidly when samples were exposed to direct light, compared to fingermark halves stored in dark conditions for the same period of time. This was partially attributed to the fact that when exposed to UV light, squalene photooxidises to volatile compounds including acetone and aldehydes [126]. This was later confirmed by Mountfort *et al*. [122]. A similar study found that the degradation rates of squalene and cholesterol on copy paper were more greatly influenced by heat and light, respectively [124]. It was noted that degradation of both compounds occurred more rapidly than reported in other studies, which was attributed to the use of standard solutions rather than latent fingermarks. Amorós *et al*. therefore proposed that latent fingermark residue acts as a protective matrix to retard degradation [124].

Increased levels of short-chain saturated fatty acids have been observed in aged fingermarks, and are thought to be derived from the degradation of these long-chain unsaturated lipids [10, 11]. Archer *et al*. reported a slight initial increase in both saturated and unsaturated fatty acids within 15 days, followed by a subsequent decrease to approximately original levels [10]. This trend occurred to a greater extent with unsaturated fatty acids when samples were stored in constant light. It has been proposed this might be due to bacterial degradation of wax esters and triglycerides to free fatty acids, which in turn are further degraded by oxidation or bacterial mechanisms or evaporate [10]. Neither Archer *et al*. nor Weyermann *et al*. observed any significant trends in long-chain fatty acid levels over a period of a month [10, 13].

6.1.2 Age estimation of latent fingermarks

The ability to estimate the age of a latent fingermark would be invaluable in instances where an individual claimed to have had contact with an item or surface related to a crime, but at a time unrelated to the incident [1, 13, 122, 161, 301]. In casework, whether or not a latent fingermark was recently deposited has been inferred from the quality of ridge development with powdering methods [161, 301]. This informal approach to estimating fingermark age has been proven to be unreliable, as high quality fingermarks up to 6 months old may be detected in such a manner [301, 303]. There have been numerous attempts to devise a method of inferring latent fingermark age, though none have been particularly successful so far, due in part to the sheer complexity of the task [13, 123, 125, 301, 304]. Environmental, inter- and intra-donor variability have frequently been cited as major obstacles in such endeavours [1, 13, 123, 125, 300, 304].

Wertheim *et al*. proposed that the measurement of chemical changes in latent fingermark composition would be the most viable approach to estimating fingermark age, rather than relying on the physical appearance of the ridge pattern [161]. Ideally, such an approach would involve monitoring a compound(s) which degrades at a rate independent of environmental factors [161]. Preliminary experiments by Duff and Menzel focused on the red-shift in laser-induced photoluminescence of uncharged fingermarks with increasing age, which was partially attributed to the photo-degradation of riboflavin [304, 305], though more recently, it has also been suggested that the red-shift is due to the formation of fluorescent protein-lipid oxidation products [125]. A fluorescence spectroscopy method developed by van Dam *et al*. was unable to be applied to fingermarks from female donors, due to insufficient amounts of fluorescent material, and was only effective on half of the samples from male donors [125].

Several approaches towards investigating fingermark degradation and estimating fingermark age directly from chemical composition using GC-MS have been explored by researchers at the University of Lausanne [13, 14, 123]. Weyermann *et al*. proposed that the ratio of the peak areas of an unstable compound (squalene) to a relatively stable compound (cholesterol) might be used construct a regression curve by which fingermark age could be estimated [13]. They also demonstrated the influence of substrate porosity on the degradation rate of squalene. Following these results, Koenig *et al*. identified specific wax ester species present in latent fingermarks as potential markers to more reproducibly determine fingermark age, and evaluated the robustness of this method when applied after latent fingermark development [14]. Girod *et al*. proposed a protocol for fingermark age estimation designed to overcome variability due to environmental and deposition factors, but acknowledged that there is currently no accepted analytical approach that could be utilised as such [123].

6.1.3 Aims

Degradation of initial sample composition due to environmental exposure, heat and oxidation processes can affect classification in chemometric models [283]. This chapter details the application of the gas chromatography-mass spectrometry method described in Chapter 5 to examine the chemical modifications undergone by latent fingermark lipids as a function of time. Principal component analysis (PCA) was performed on this data to assess the effects of inter-donor variation and storage conditions on the types and rates of degradation processes.

6.2 Experimental

6.2.1 Reagents

Dichloromethane (≥99.9 %; Macron Chemicals, USA) was used as received.

6.2.2 Sample collection and storage

Latent fingermark samples were collected from 8 donors, using the 'double sampling' protocol as outlined in Chapter 5. A summary of the characteristics of the donor population is presented in Table 6.1. Fourteen samples were collected in triplicate from each donor over the course of 5 hours to provide a total of 336 fingermarks. A maximum of 5 samples were collected at a time, with a period of at least 1 hour in between sampling times, to allow sebum to re-accumulate on the skin surface. Information regarding donor activity and handled substances was collected at each sampling time.

Table 6.1: Summary of donor information

Sex	*n*	Age (years)	*n*	Recent skin product use	*n*
Male	4	20-29	6	Yes	7
Female	4	30-39	2	No	1

One sample from each donor was analysed by GC-MS on the day of collection (within 2 hours of deposition) to obtain profiles of initial fingermark composition. 12 samples from each donor were placed in uncapped 20 mL glass vials (Gerresheimer, Germany). Replicate samples were stored in individual vials and bundled together with rubber bands. Sample vials were placed in a tray and stored on a shelf in an office environment at room temperature (21 – 23 °C) with exposure to light and airflow, for up to 28 days (Figure 6.1). An open vial containing clean filter papers was stored with the samples to provide analytical blanks for each analysis time. The final samples from each donor were stored in glass vials that were completely wrapped in aluminium foil, and stored in a cardboard box adjacent to the open-topped vials. These samples were stored alongside the samples in open vials for 28 days.

Figure 6.1: Arrangement of latent fingermark samples stored in open vials over a 28 day period

6.2.3 Sample preparation

Sample preparation for GC-MS was conducted as outlined in Chapter 5. From the samples stored in open glass vials, one randomly chosen sample from each donor was analysed 0, 2,

5, 7, 9, 12, 14, 16, 19, 21, 23, 26 and 28 days after deposition. The samples stored in foil-wrapped vials were analysed 28 days after sample collection.

6.2.4 Chemical analysis

Chromatographic analysis was performed using a 6890N series GC interfaced with an Agilent 5975 inert mass selective detector. A 1 µL aliquot was introduced into the split/splitless injector by means of a Gertsel MPS2 autosampler. The gas chromatograph was fitted with a Phenomenex ZB-5MS 30 m x 0.25 mm ID x 1 µm d_f column.

6.2.5 Analytical conditions

Analytical conditions were as described in Chapter 5.

6.2.6 Data analysis

Pre-processing of the data and principal component analysis (PCA) was carried out as outlined in Chapter 5. Distance plots were constructed using Microsoft Excel.

6.3 Results and discussion

6.3.1 Compositional changes over time

The work of Mong *et al*. [11] and Archer *et al*. [10] remain the most extensive investigations into latent fingermark degradation. A crucial aspect missing from these studies is a frequent and consistent monitoring of compositional changes, to establish whether degradation rates are uniform under constant environmental conditions, and how degradation processes may vary between donors. Mong *et al*. analysed samples from all donors at infrequent intervals of 0, 10, 30 and 60 days after deposition (or 0 and 30 days in the case of children's samples) [11], while Archer *et al*. analysed samples at smaller time intervals of 1 – 12 days over 33 days, but samples from only up to three of the five donors were analysed at any one time [10]. Therefore, analysis of samples from all donors was conducted every 2 – 3 days throughout the 28 days of this study, in order to determine at what period after deposition samples no longer classified with those analysed on the day of deposition.

The 8 donors sampled in this investigation were selected based on the chromatograms of fingermark samples provided in the inter-donor variability investigation described in Chapter 5, as well as for the relatively small difference in age. A variety of good and poor donors were chosen, as well as some known to use cosmetic products regularly. The initial design for this experiment included the incorporation of prepubescent donors, as extensive

studies into the degradation of children's fingermarks using GC-MS have not previously been reported. Due to difficulties in analysing children's fingermarks (discussed in Chapter 5), this was ultimately not carried out.

Major compositional changes with increasing sample age were found to be common to all donors concerning the relative abundance of the 15 compounds of interest during the 28 day period. The most obvious change was the marked reduction in peak height of squalene, such that hexadecenoic acid and palmitic acid became the predominant compounds (Figure 6.2). The precise timing and the extent of this change appeared to be dependent on the initial composition of the fingermark; samples collected from donors with a naturally low fatty acid to squalene ratio exhibited this change sooner than those from donors with a much higher ratio. It should be noted that retention times can change through ageing of the column, due to loss of the stationary phase by depolymerisation, hence the decrease in retention times observed throughout this study [120, 290].

The rapid diminishing of squalene is consistent with observations made by Archer *et al*. [10], with the exception that squalene was still detected in samples from 7 of the 8 donors after 28 days, whereas Archer *et al*. reported that squalene could not be detected in any samples stored under constant light after 20 days. The experimental conditions used by Archer *et al*. utilised constant, direct illumination to contrast with the effect of storage in complete darkness, whereas here, samples were stored under fluorescent office light set to switch off when the office was unoccupied, thereby providing a more typical diurnal exposure. Samples were stored in an office environment rather than a laboratory to mimic the conditions on which fingermarks on documents or other paper substrates might commonly be stored, and to prevent contamination from reagents [11]. This difference in illumination conditions may account for the differences in squalene degradation, given that squalene undergoes photo-oxidative degradation [126]. Alternatively, Amoros *et al.* have postulated that the degradation of squalene is affected less by exposure to light than elevated temperatures [124]. In this case, the use of an incandescent light bulb by Archer *et al*. may also have contributed to thermal degradation of fingermark constituents (storage temperature was reported as 25 °C, compared to 21 – 23 °C in this investigation), thereby accelerating squalene degradation.

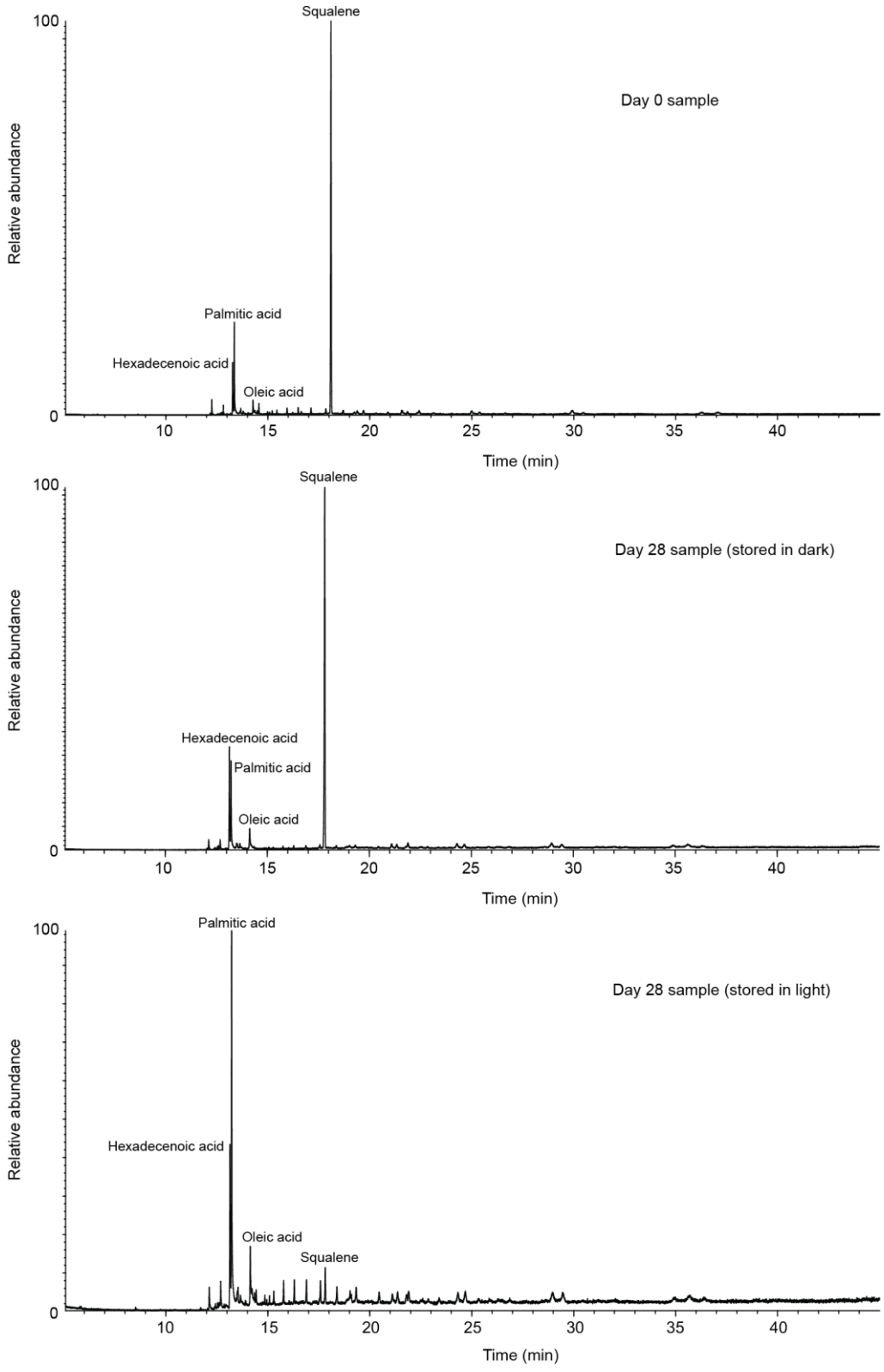


Figure 6.2: Total ion chromatograms representing changes in relative abundances of compounds detected in fingermarks from a single donor

The peak areas of the free fatty acids themselves showed scatter, with a general trend towards an overall increase at the end of the 28 days. Similarly, the proportion of the peak

areas of the saturated wax esters to their monounsaturated counterparts appeared to increase. It is unclear from only a visual inspection of the chromatograms as to whether these represent actual compositional changes related to the age of the fingermark [11]. The destructive nature of the extraction and GC-MS method necessitates the assumption that all samples from each donor have an identical initial composition, but as demonstrated in Chapter 5 and published studies, latent fingermarks can exhibit significant intra-donor variation, which impacts upon reproducibility. GC-MS studies into the ageing of latent fingermarks, particularly age estimation, that utilise absolute quantification methods are frequently complicated by difficulties in obtaining reproducible, homogenous samples for comparative purposes [10, 11, 123, 125].

PCA of the total dataset (329 chromatograms) revealed that 99.23 % of the variance within the dataset was accounted for by the first 5 PCs (Figure 6.3). Based upon the Scree plot, it can be seen that up to 4 PCs can be used to model the data, as the fifth PC only describes 0.37 % of the variance. Scores plots were generated using a variety of combinations of the first 4 PCs. The use of the fourth PC did not lead to any further discrimination, which is not unexpected as it described only 1.36 % of the variance.

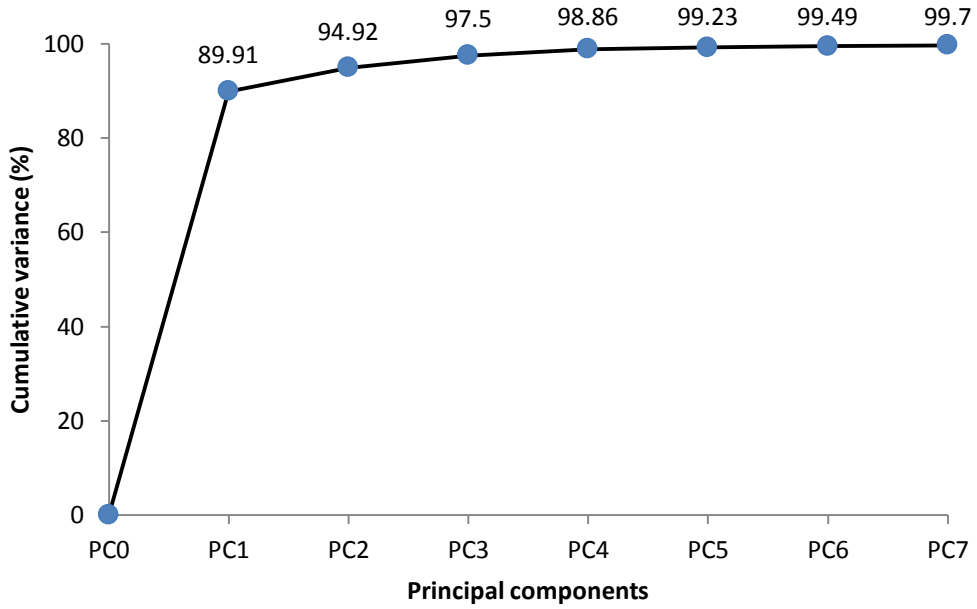


Figure 6.3: Scree plot depicting the variance in the dataset accounted for by each PC

The scores plot constructed from the first 3 PCs (Figure 6.4), revealed significant changes in composition occurred over the 28 days of the investigation. 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 compositional differences as discussed in Chapter 5. Over the 28 day period, samples from each of the eight 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.

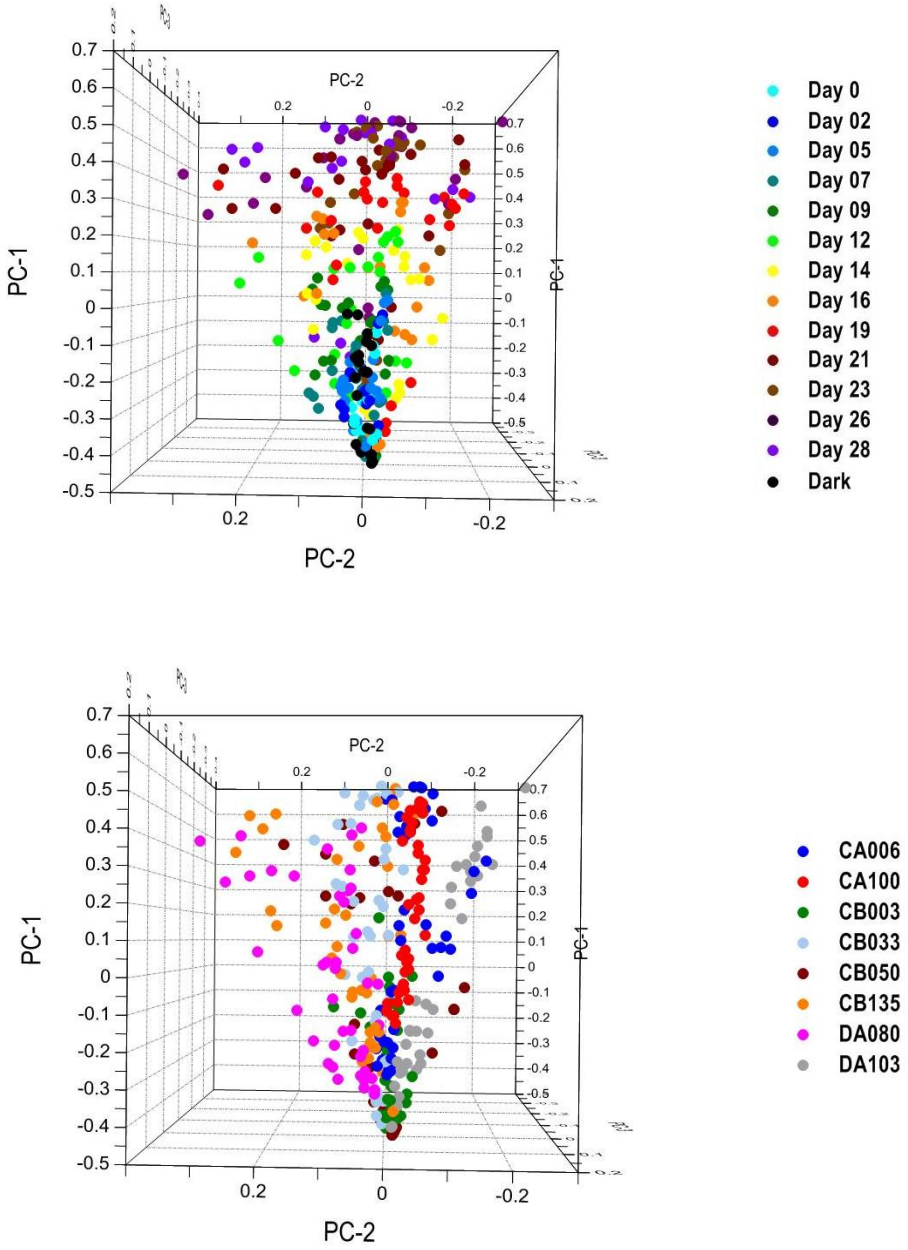


Figure 6.4: 3-dimensional scores plot generated from the first 3 PCs, demonstrating the distribution of fingermarks of increasing age collected from 8 donors. Samples are colourised by sample age in days (top) and individual donors (bottom)

The factor loadings for the first 3 PCs (Figure 6.5) were utilised to identify the compounds that contributed to the variance within the dataset. The loadings plot for PC1 revealed significant negative correlation to squalene, as well as some positive correlation to palmitic acid. More recently deposited samples, which contained relatively large relative abundances of squalene, and correspondingly low amounts of palmitic acid, attained negative scores on PC1, while older samples had increasingly positive scores on PC1 as squalene degraded. The factor loadings for the first PC are almost identical to those of the first PCs discussed in Chapter 5. In this case, the relative amounts of squalene and palmitic acid 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 cannot be used to monitor changes in fingermark composition as a function of time. The loadings plot for PC2 revealed significant negative correlation to palmitic acid, as well some positive correlation to cholesterol. The loadings plot for PC3 revealed significant negative correlation to cholesterol, significant positive correlation to hexadecenoic acid, and some negative correlation to palmitic acid. 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 second and third PCs is due to sample age, inter-donor variation or a combination of the two. The factor loadings of these PCs may be similarly reflective of both inter-donor and age-related compositional differences.

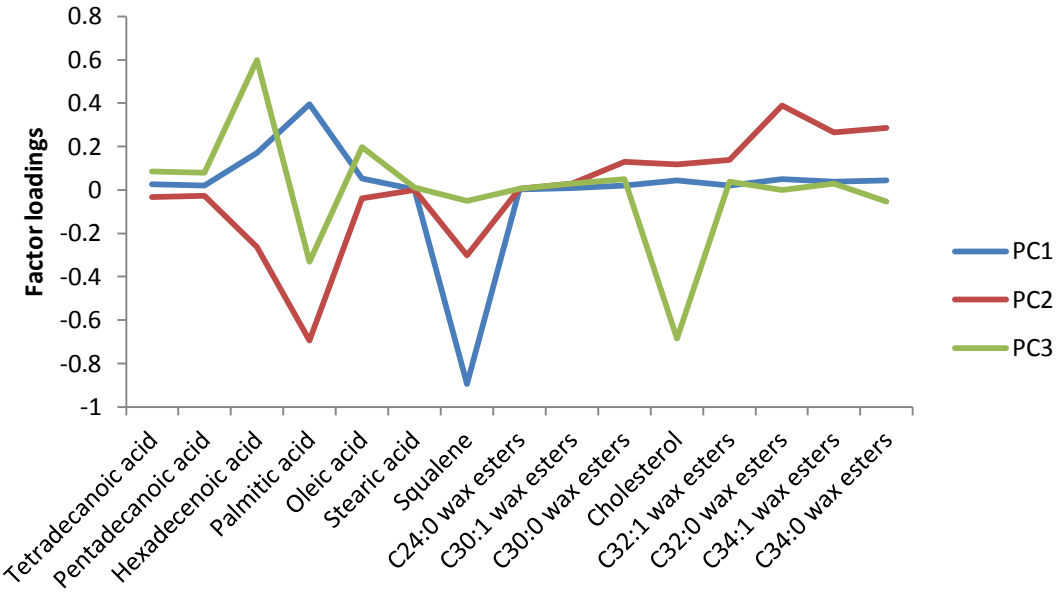


Figure 6.5: Factor loadings plots for the first 3 PCs

The degradation of sebaceous lipids over time has a marked impact on the ability to classify latent fingermarks as belonging to a particular individual or indicating characteristics. With increasing age, samples from the total dataset are projected in a scatter, rather than forming separate groups. While the scatter of 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 widespread. The inconsistency of fingermark degradation may pose enormous difficulties in establishing a method of estimating fingermark age, but does demonstrate that lipid degradation can be expected to significantly affect any kind of classification model.

6.3.2 Effect of storage conditions on degradation rate

Storage conditions appeared to have a significant impact upon squalene degradation, as samples stored in complete darkness for 28 days did not exhibit the dramatic decrease of this compound seen in the samples stored in open vials for the same length of time (Figure 6.2). Samples that had been stored in foil-wrapped vials for the duration of the investigation were subsequently projected very close to the day 0 samples for each donor in the PCA scores plot (Figure 6.4). This observation is consistent with previous reports that the degradation of squalene in latent fingermarks is accelerated by exposure to light, compared to those stored in dark conditions [10, 122]. Due to time and practicality constraints, additional samples were not collected to further investigate the effects of sample storage conditions on degradation rate throughout the 28 day period.

While it has been suggested that the age of a latent fingermark may be estimated from its chemical composition, it is shown here that the environment that a fingermark has been exposed to, including factors such as exposure to light and airflow, has a significant effect on the rate of degradation of certain compounds. The effects of other factors such have temperature and humidity have been speculated upon, but not as thoroughly investigated [10, 124]. Additionally, substrate type has been shown to have a marked effect on fingermark longevity, with faster degradation on nonporous substrates than porous ones [13].

6.3.3 Inter-donor variation

As discussed above, the disparate projection of samples from the 8 donors caused by inter- and intra-donor variation, as well as sample age, creates difficulties in interpreting the dataset in its entirety. The extent to which samples from each donor are projected along PC1 in the scores plot generated from the total dataset indicates that the rate of change in

fingermark composition over the 28 day period is also subject to inter-donor variation. Subsequently, samples from each donor were treated as individual datasets to better enable examination of compositional changes as a function of time and storage conditions, independent of inter-donor variables.

PCA of samples from each donor revealed that the first two PCs accounted for almost all of the variance (≥93 %) within each dataset (Figure 6.6). PCA data for one donor (CA006) is provided in this section as an example of the results discussed in this section. Data for the remaining donors are included in Appendix 3. Based upon the Scree plot, it can be seen that up to 3 PCs can be used to model the data. Scree plots of samples from other donors showed that only 2 PCs were appropriate. For consistency, all scores plots were generated from the first 2 PCs.

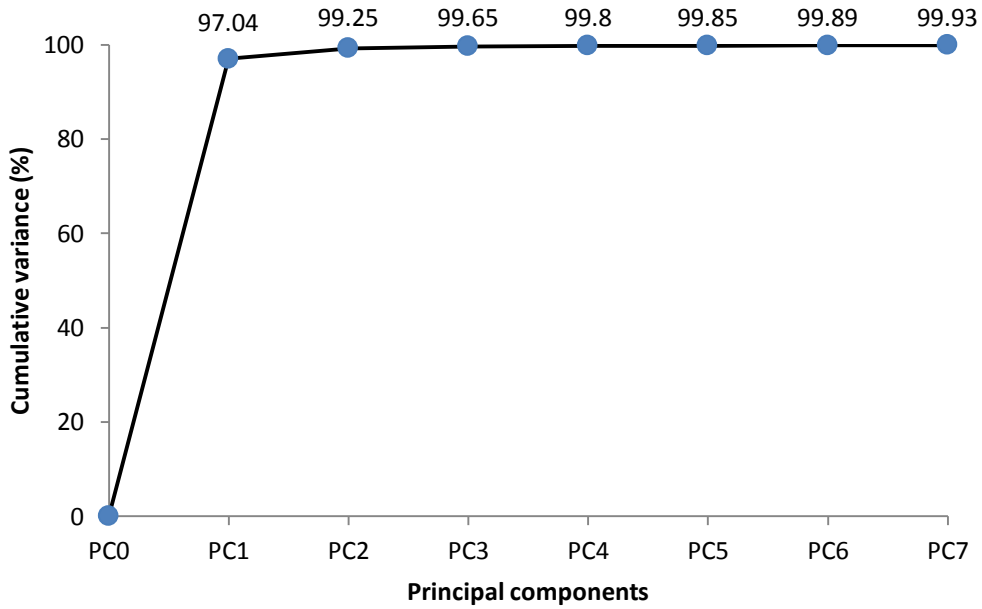


Figure 6.6: Scree plot depicting the variance in samples from donor CA006 accounted for by each PC

The scores plots constructed from the first 2 PCs (Figure 6.7), were broadly similar to the scores plot generated from the total dataset. Samples were projected primarily along the first PC, with older samples again attaining increasing scores. When fingermark degradation was examined on a per donor basis, other compositional changes became more evident. Examination of the scores plots of the first two PCs revealed that samples from each donor appeared to exhibit a 'stable period', in which older samples were projected relatively close to the day 0 samples. In most donors, this period lasted approximately one week following deposition; however, a range of 2 – 12 days was observed in some donors. This indicated

that lipid degradation processes and rates vary between individuals, which is in part a reflection of the initial starting composition. Similarly, additional groups 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 complete darkness were generally projected close to samples analysed within up to a few days following deposition.

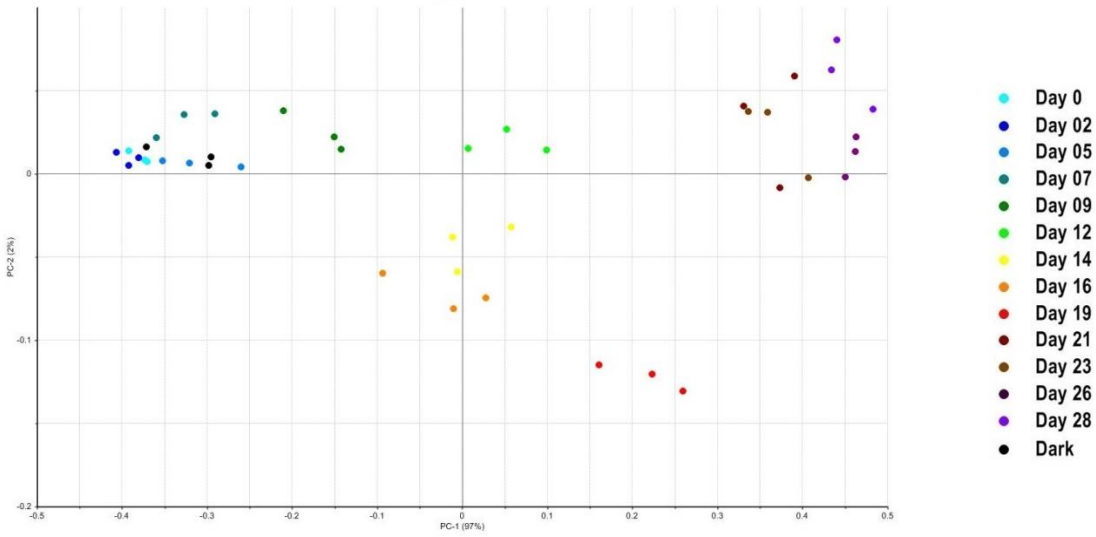


Figure 6.7: 2-dimensional scores plot generated from the first 2 PCs, demonstrating the distribution of fingermarks of increasing age of samples from donor CA006

The factor loadings for the first 2 PCs were utilised to identify the compounds that contributed to the variance within each dataset (Figure 6.8). Consistent with section 6.3.1, the loadings plots for PC1 revealed significant negative correlation to squalene, emphasising the influence of squalene degradation as a predominant degradation process. The loadings plots for the second and third PCs, revealed different chemical changes between donors, mainly fluctuations in the relative amounts of free fatty acids, with some influence from cholesterol and saturated wax esters in some donors. In some donors, there appeared to be a decrease in fatty acids, followed by an increase over the 28 days. In other donors, the reverse was seen. Archer *et al.* similarly observed that the amounts of long chain fatty acids first decreased, and then increased, 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 [10]. 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 [10]. Conversely,

Weyermann *et al*. reported no significant changes in fatty acid concentrations over 30 days [13], however these samples were stored in complete darkness.

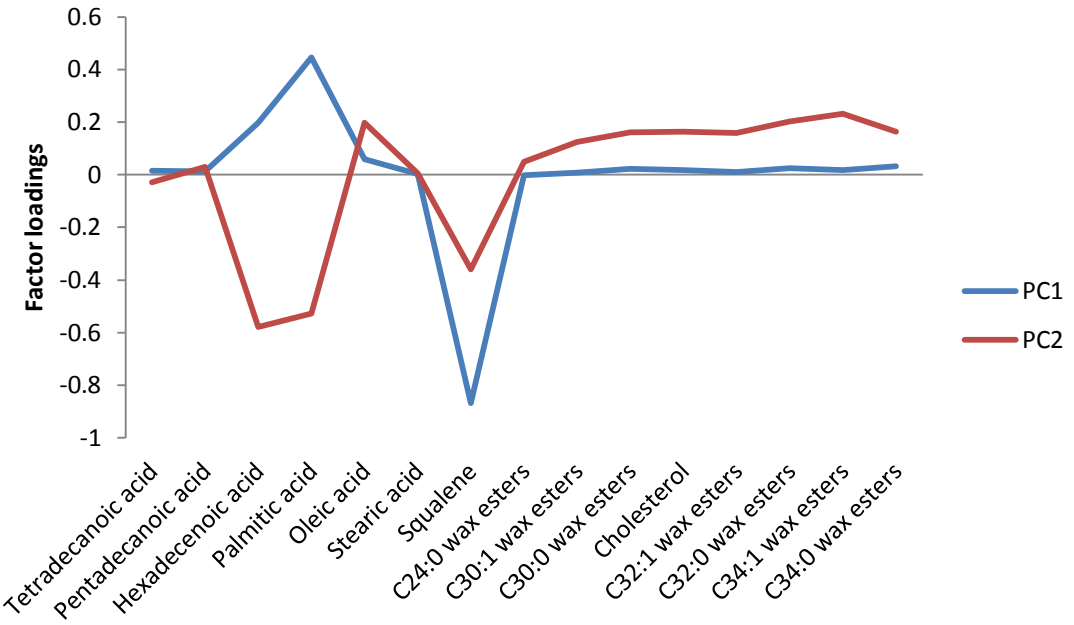


Figure 6.8: Factor loadings for the first 2 PCs of samples from donor CA006

The rate and nature of fingermark degradation, as well as differences in rate between donors, were further investigated using distance plots. These were constructed from the datasets from each donor using the scores from the first two PCs as x, y coordinates for each sample. The centroid (i.e. the mean coordinates) of the day 0 replicates was used as the point of origin for each distance plot ($\bar{x}, \bar{y}$), and the distances between each of the samples and the centroid were calculated using the formula:

$$distance\ (d) = \sqrt{(x - \bar{x})^2 + (y - \bar{y})^2}$$

Fingermarks from all donors that were stored in the light followed a general trend of increased distance from the centroid with time, which for five of the donors appeared to be linear (Figure 6.9). 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 eight 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 the light.

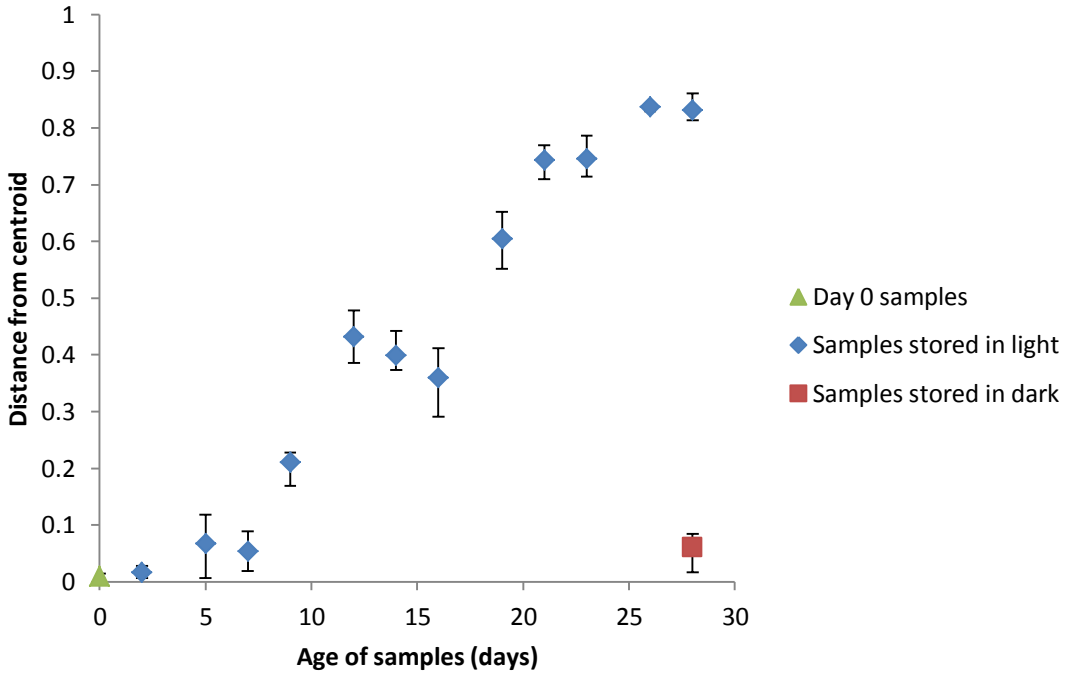


Figure 6.9: Distance plot constructed from scores of first two PCs, depicting rate of total compositional change over time of samples from donor CA006

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 [10, 11]. 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 [123]. 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 [123]. Secondly, as demonstrated in this chapter 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. A similar method proposed by Baniuk appears to rely on the assumption that the storage conditions can be reproduced accurately from observations at the crime scene, and have remained static until this time [300]. Given the impact 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 [10].

The main implication of these results is that any model used to estimate fingermark age will be limited insofar as accounting for all the variables which may affect the rate of degradation. It may be that several models would be required to account for individual environmental factors. Exposure to varying degrees of light, temperature, humidity, immersion in water (which retards oxidation of 'fragile fraction' lipids), airflow, microbial action, and presence of contaminants are only some of the factors which can affect lipid degradation. While such methods would be of limited use to forensic investigations, they may assist in providing detailed information regarding fingermark degradation processes and possible new target compounds in degradation products.

6.4 Conclusion

The gas chromatography-mass spectrometry method outlined in Chapter 5 was successfully used to monitor changes in the relative abundances of latent fingermark lipid components over a 28 day period following fingermark deposition. It was established that changes in fingermark composition with time had a significant impact on the projection of samples within a PCA scores plot, in comparison to samples analysed on the day of deposition. This was attributed principally to the degradation of squalene.

A PCA scores plot of the entire dataset demonstrated that inter-donor variation had a significant impact on the distribution of samples at all analysis intervals. Additionally, PCA and distance plots showed the rate of compositional changes with time varied greatly between donors. Difficulties in obtaining reproducible samples from individual donors further complicated the distribution of degraded samples within scores plots, such that fingermarks could not be reliably classified by age.

A preliminary comparison into the effects of storage conditions showed that exposure to light had a significant impact on the photo-oxidation rate of squalene, with samples stored in the dark for 28 days often exhibiting little difference from the initial fingermark composition. While only a limited number of environmental conditions were explored, the results obtained in this investigation reinforce that there are many challenges facing the development of fingermark classification models, as well as means of estimating latent fingermark age.

Chapter 7: Conclusions and future work

This thesis has outlined the significance of the continued studies into fingermark development techniques, as well as latent fingermark composition, for both fingermark detection and the possible construction of classification methods based upon compositional differences. The aims of this project were to investigate the lipid fraction of latent fingermarks, in both the development of novel detection methods and chemical analysis. The issues faced in all aspects of latent fingermark research have been discussed with particular regard to difficulties in obtaining reproducible samples, analytical constraints and the lack of standardised methodology.

It has been demonstrated that there are clear differences in the detectability and associated chemical composition of fingermark lipids as functions of donor traits and sample age. However, 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. In addition, it was shown that efforts to estimate fingermark age based on lipid composition is problematic due to the influences of inter-donor variation and storage environment on chemical changes as a function of time.

In addition to the specific conclusions at the end of each chapter, suggestions for future work are discussed below.

7.1 Histological stains as fingermark development reagents

Chapter 3 discussed the potential of two modified histological stains, Oil red O in propylene glycol and aqueous Nile blue, for latent fingermark development on both porous and non-porous substrates, as less hazardous alternative formulations to similar, reported reagents. It was demonstrated that both Oil red O and Nile blue are effective for the development of charged latent fingermarks on a variety of substrate types. These simple and inexpensive reagents therefore have potential for operational fingermark detection in conjunction with existing methods such as physical developer. More importantly, Nile blue is one of only a few lipid-specific photoluminescent reagents, which is a great advantage over current operationally-used methods for porous substrates.

There are some issues that must be addressed to satisfy the requirements outlined in the International Fingerprint Research Group's guidelines, for either of these reagents to be considered recommendable for operational use by the wider fingermark research community. Nile blue particularly is yet to be fully evaluated. Further work is required to

optimise the formulation of this reagent, as well as to compare the performance of Nile blue with other lipid-sensitive fingermark reagents, such as Oil red O and physical developer. Additionally, the performance of Nile blue on a wider range of substrate types, uncharged and older fingermarks, needs to be more extensively investigated to determine the full capabilities of this method.

The work presented in Chapter 4 goes some way towards illustrating the variation in latent fingermark development that might be expected from 'real' fingermarks encountered in criminal investigations. It was demonstrated that while Oil red O was largely ineffective on uncharged latent fingermarks, physical developer performed well in comparison on both recently deposited and 1 month old samples. Additionally, physical developer performance was found to show significant variation as a function of donor traits. Furthermore, the large body of data generated by the donor study may be amenable to further statistical analyses in the future. There is also potential to conduct additional studies targeted to specific traits, including those not examined in this thesis, such as diet and ethnicity.

7.2 Practical considerations for large-scale studies

The investigations carried out in Chapters 4 and 5 revealed the inter-donor variability of latent fingermark lipids via both development reagents and compositional analysis. The donor populations sampled from in these investigations were significantly larger than many of those in other reported studies, enabling the data obtained to be more representative of a wider, general population.

This work also demonstrated the difficulties in obtaining fingermarks from a donor population of considerable size (in excess of 100 individuals) and of varied ages. It is recognised that there is a need for compromise between obtaining sufficient numbers of samples to produce statistically valid data and practicality in experimental design. The time constraints imposed by the duration of this project limited the number of donors that could be sampled from, thus only a small number of donor characteristics (age, sex, use of skin products) were discussed in the context of this data.

Ongoing research into compositional variation may instead benefit from targeted studies focused on specific donor traits. Planning and organisation of sample collection from a representative subset of a given population requires cooperation from a number of educational and care organisations, particularly in regards to prepubescent, adolescent and elderly fingermark donors, and this may necessitate longer periods of time for both

collection and analysis than originally anticipated. Additionally, ethical considerations and restrictions may vary between institutions, and this must be considered together with the aims of the intended research.

7.3 Instrumental considerations

Chapter 5 described the development of a simple gas chromatography-mass spectrometry (GC-MS) method for the detection of a number of fingermark lipids. Analysis of samples from a large donor population showed that the relative amounts of squalene and free fatty acids accounted for the vast majority of compositional variation between donors. However, there was 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. Before such an approach to profiling latent fingermark composition can be discounted, it must be noted that the method described in this thesis is exploratory in nature and there is potential for improvement. The inclusion of a greater number of endogenous compounds into a classification model would greatly improve discrimination between samples. The developed GC-MS method is limited in its detection of wax esters and is unable to be applied to triglycerides. The relative amounts of triglycerides and free fatty acids, one of the greatest sources of variation in sebum composition, is a potential source of variability in latent fingermarks that is yet to be explored. The use of a GC column that is stable at higher temperatures than those employed in this project would be greatly beneficial to the separation of all classes of sebaceous lipids, and may provide additional information pertinent to classification of fingermark composition. Alternatively, a liquid chromatography method may be more amenable to the separation and characterisation of wax esters and triglycerides.

The imperfect nature of latent fingermarks presents great complications to the proposition that chemical composition could be used for identification or dating purposes. Variation in the way donors deposit fingermarks will often result in the formation of incomplete or partial ridge patterns, which limits analysis to relative quantification of lipid components. Fingermarks from children and other poor lipid donors were difficult to detect using the developed GC-MS method. Uncharged fingermarks, which may be more representative of those deposited by incidental contact at crime scenes, are likely to be similarly incompatible with the presented method. Any approach for analysing fingermark composition for the purposes of criminal investigations would be frequently ineffective

unless it was sufficiently sensitive to detect material from partial fingermarks. Employing a concentration step by evaporating excess solvent from the sample extracts, or preparation of more volatile derivatives could improve detection by GC-MS in these instances.

Chapter 6 described the application of the GC-MS method developed in Chapter 5 to monitor changes in fingermark samples as a function of time. It was shown that the principal change in the composition of samples stored with exposure to light over 28 days was the degradation of squalene. Inter-donor variation was found to impact upon sample projection within a PCA scores plot such that classification of samples by age was not possible using either the entire donor population to construct such a model. The destructive nature of GC-MS necessitated the experimental setup described in Chapter 6, but introduced problems into the interpretation of the data related to intra-donor variation. Mass spectrometry imaging (MSI) may be a more suitable approach for such studies, as it can monitor composition changes over time *in situ*, rather than requiring the extraction and destruction of the fingermark. A portion of this thesis was originally intended to employ matrix-assisted laser desorption/ionisation (MALDI) MSI in this regard, as while the technology is still at a developmental stage, comparisons to GC-MS may provide complimentary information. While logistical issues ultimately prevented this work from being carried out, this approach retains great potential as a means to explore latent fingermark composition.

7.4 Factors affecting fingermark degradation

The sheer quantity 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. The results obtained in Chapter 6 emphasise that storage conditions can have significant effects on the rate of degradation. Unfortunately, practicality issues regarding the number of samples that could be monitored limited the environmental conditions that were explored to a brief comparison. The analytical investigations described in this thesis have shown only preliminary results, and further work will be required to fully explore the potential of utilising fingermark composition to enable the development of classification systems for donor traits or individualisation and fingermark age. All of the samples in this investigation were stored in identical conditions, barring exposure to light and airflow. The effect of temperature is an important factor that needs to be explored further, particularly considering the significant effect that higher temperatures have on the degradation of children's fingermarks. A large-

scale experiment similar to the University of Tennessee's body farm, whereby samples are exposed to a wide range of environmental conditions, would be required to obtain a full understanding of the effects of storage on fingermark degradation.

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Appendices

Appendix 1: Donor participation forms (see 2.2.1)

Curtin University

Department of Chemistry

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Participant Information Sheet

Our names are Patrick Fritz and Amanda Frick. We are currently conducting a research project as part of our PhD (Chemistry) degrees at Curtin University of Technology.

Purpose of Research
We are investigating the chemistry of latent fingermarks using chemical imaging and fingermark development techniques. This constitutes part of an on-going program of research at Curtin into the chemical composition of latent fingermark residue, the outcomes from which will provide a basis for improvements to existing detection techniques and the development of new methods.

How You Can Help
This research depends upon a large collection of sample latent fingermarks to analyse. We are requesting you allow the research team to collect a sample of your fingermarks for this research. The fingermarks will be collected on a variety of surfaces including paper and polished metal plates. Information concerning your age, gender and history of recent activity at the time of sample collection will also be collected.

Consent to Participate
Your involvement in the research is entirely voluntary. You have the right to withdraw at any stage without it affecting your rights or my responsibilities. When you have signed the consent form we will assume that you have agreed to participate and allow us to use your data in this research.

Confidentiality
The fingermarks you provide will be allocated a number and kept separate from your personal details. It will not be possible for the impression to be associated with you by any outside person. In adherence to Curtin University policy, the impression and separate information will be kept in a locked cabinet within the Chemistry Department at Curtin for at least five years, before a decision is made as to whether it should be destroyed.

Further Information
This study has been approved by the Curtin University Human Research Ethics Committee (Approval Number SMEC-07-13). If needed, verification of approval can be obtained either by writing to the Curtin University Human Research Ethics Committee, c/-Office of Research and Development, Curtin University of Technology, GPO Box U1987, Perth, 6845 or by telephoning 9266 2784. If you would like further information about the study, please feel free to contact us by email (patrick.fritz@postgrad.curtin.edu.au or amanda.frick@postgrad.curtin.edu.au) or by phone (Patrick Fritz: 0434055875, Amanda Frick: 0439525913). You can contact our supervisor Professor Simon Lewis on 9266 2484 or s.lewis@curtin.edu.au.

Thank you very much for your involvement in this research.

Your participation is greatly appreciated.

Appendix 1.1: Information sheet provided to fingermark donors

Curtin University

Department of Chemistry

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CONSENT FORM

- I understand the purpose and procedures of the study.
- I understand that the procedure itself may not benefit me.
- I understand that our involvement is voluntary and we can withdraw at any time.
- I understand that no personal identifying information like names and addresses will be used in any published materials.
- I understand that all information will be securely stored at Curtin for at least 5 years before a decision is made as to whether it should be destroyed.
- I have been given the opportunity to ask questions about this research.
- I agree to participate in the study outlined to me.

Name: _____

Signature: _____

Date: _____

Appendix 1.2: Consent form filled out by adult fingermark donors

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PARENTAL CONSENT FORM

- I understand the purpose and procedures of the study.
- I understand that the procedure itself may not benefit me or my child.
- I understand that our involvement is voluntary and we can withdraw at any time.
- I understand that no personal identifying information like names and addresses will be used in any published materials.
- I understand that all information will be securely stored at Curtin for at least 5 years before a decision is made as to whether it should be destroyed.
- I have been given the opportunity to ask questions about this research.
- I agree to allow my child to participate in the study outlined to me.

Child's name: _____

Parent/guardian's name: _____

Parent/guardian's signature: _____

Date: _____

Appendix 1.3: Consent form filled out by parents/guardians of fingermark donors under 18 years of age

Donor number:

Age (years):

Gender:

Date of collection:

Washing of hands (< 1hr):

Y - N

Food handling (< 1hr):

Y - N

Washing of hands since handling food:

Y - N

Recent use of cosmetics/skin care products (within 12hrs):

Y - N

Recent use of cosmetics/skin care products (within 24hrs):

Y - N

Recent handling of any other greasy/dirty substances within 12 hrs (please describe if yes):

Y - N

_____

Appendix 1.4: Questionnaire filled out by fingermark donors

Appendix 2: Statistics (see 4.3.2.3)

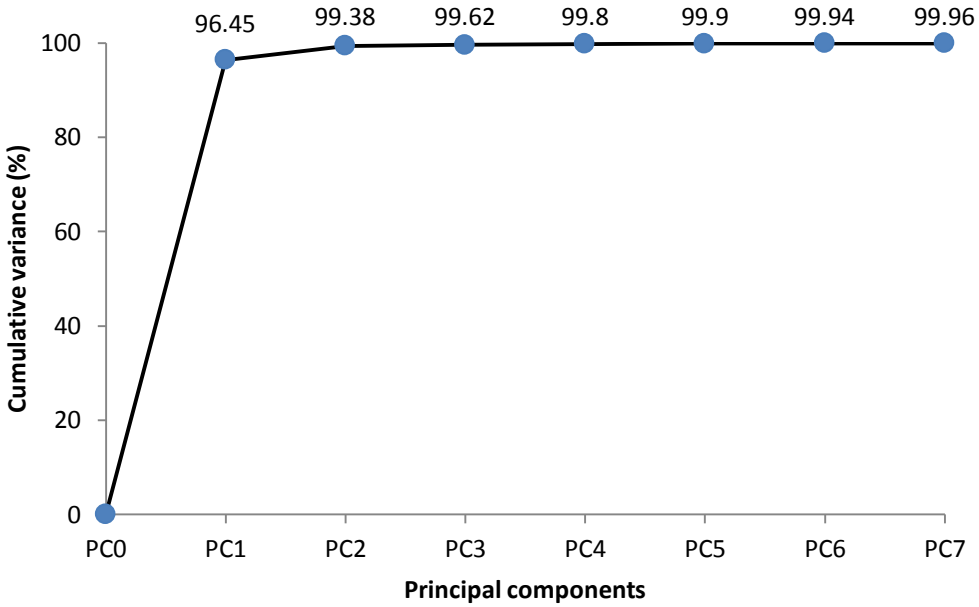
Appendix 2.1: Statistical values obtained from Mann-Whitney *U* tests of median grades given to samples treated with PD within 36 hours, as a function of donor sex and skin product use

	Recent skin product use (12 hours)		No recent skin product use		Female donors		Male donors	
	Female	Male	Female	Male	Recent skin product use	No recent skin product use	Recent skin product use	No recent skin product use
Donors (*n*)	39	23	32	54	39	32	23	54
Median	1	1	1	1	1	1	1	1
Mean	1.64	1.43	1.19	0.89	1.64	1.19	1.43	0.89
Standard deviation	1.11	0.95	0.93	1.09	1.11	0.93	0.95	1.09
U score	675.5		673.5		495		398	
Z-score	-0.27		-1.80		-0.03		-2.63	
p-value	0.79		0.07		0.98		0.01	

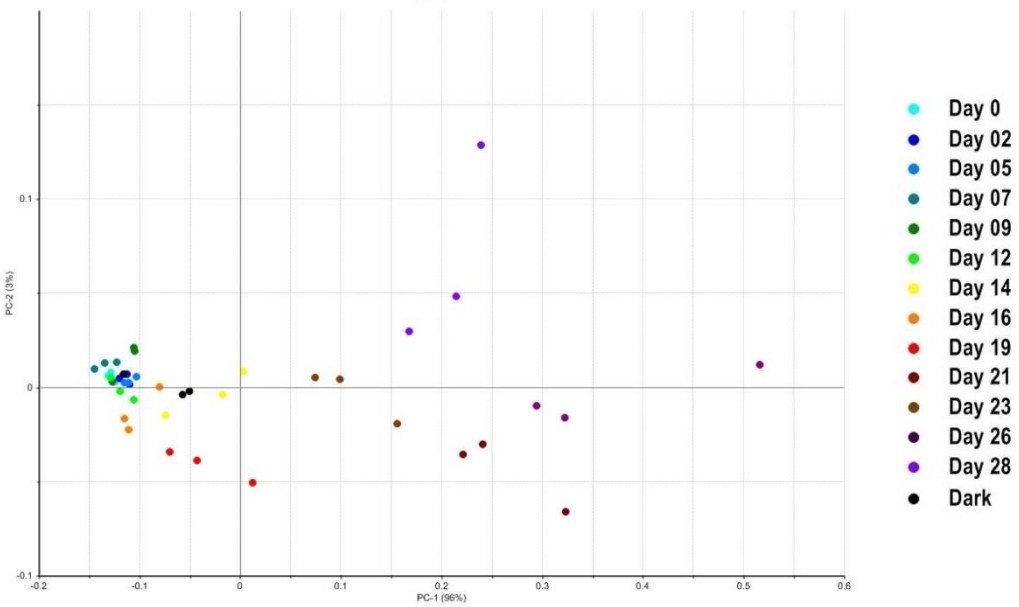
Appendix 2.2: Statistical values obtained from Mann-Whitney *U* tests of median grades given to samples treated with PD within 36 hours, as a function of donor age and skin product use

	Recent skin product use (12 hours)		No recent skin product use		Under 25 years old		25 and over	
	Under 25 years old	25 and over	Under 25 years old	25 and over	Recent skin product use	No recent skin product use	Recent skin product use	No recent skin product use
Donors (*n*)	22	40	47	39	22	47	40	39
Median	1	1	1	1	1	1	1	1
Mean	1.64	1.53	0.81	1.23	1.64	0.81	1.53	1.23
Standard deviation	1.18	0.99	1.01	1.04	1.18	1.01	0.99	1.04
U score	424		680.5		296		661	
Z-score	-0.25		-2.16		-3.00		-1.23	
p-value	0.80		0.03		0.003		0.22	

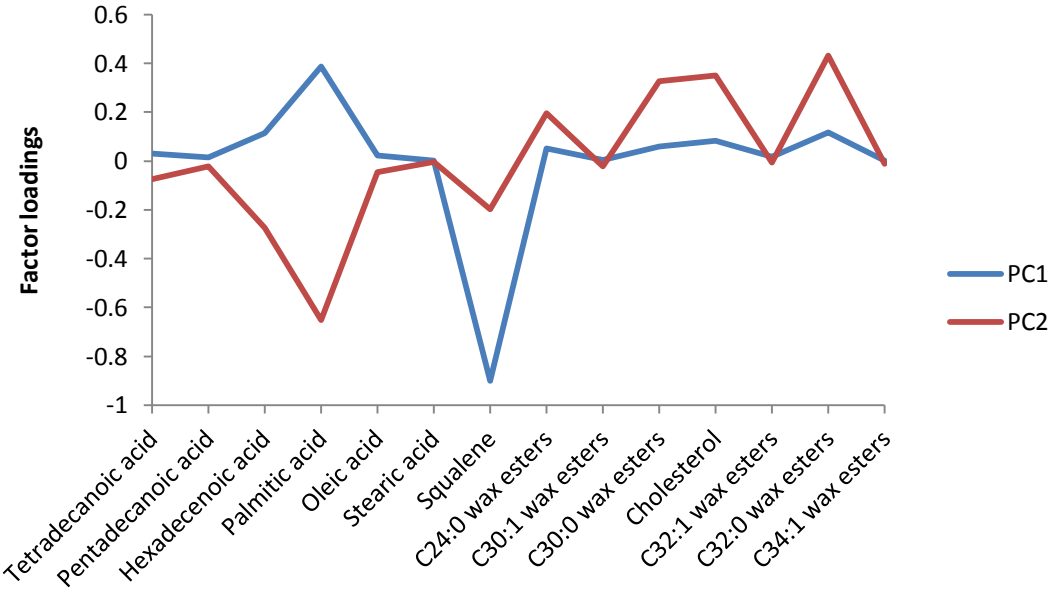
Appendix 3: Fingermark degradation (see 6.3.3)



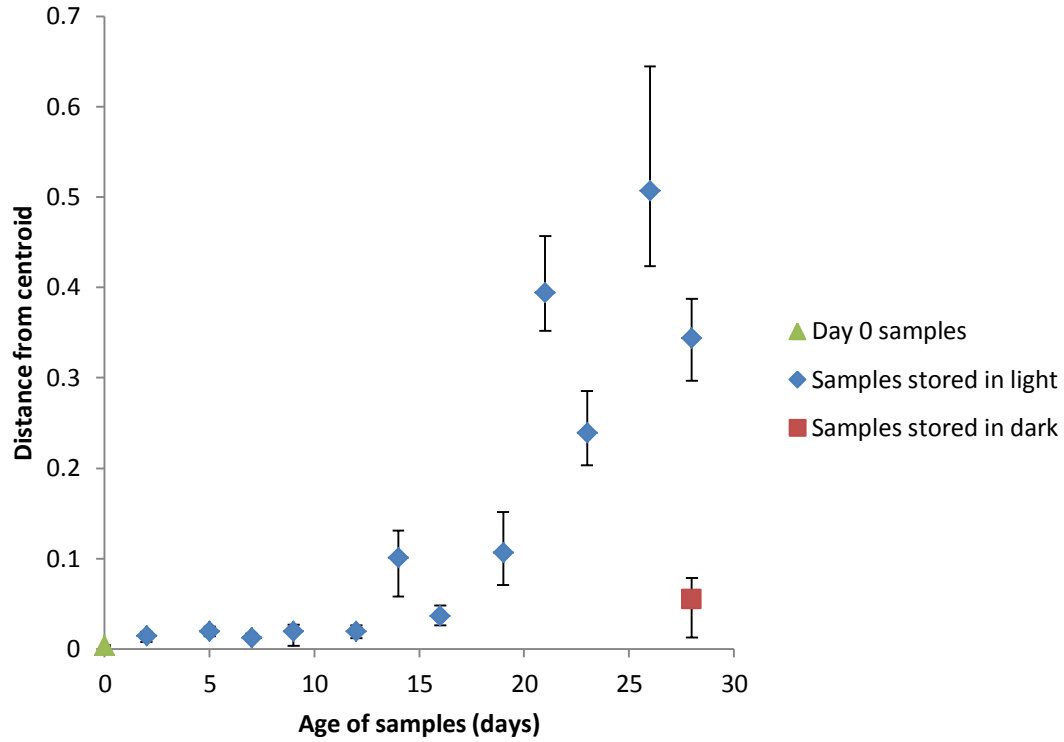
Appendix 3.1: Scree plot depicting the variance in samples from donor CB003 accounted for by each PC



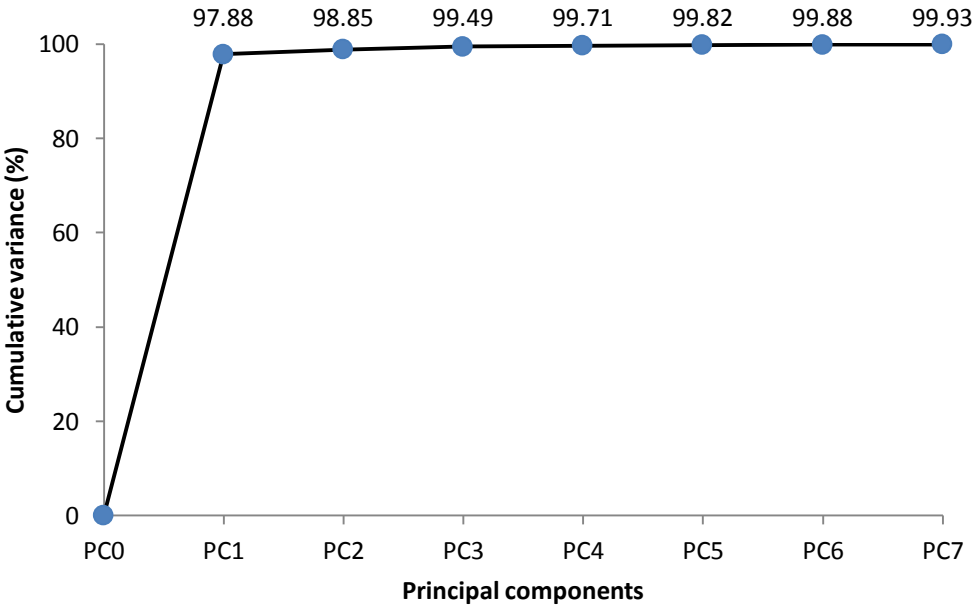
Appendix 3.2: 2-dimensional scores plot generated from the first 2 PCs, demonstrating the distribution of fingermarks of increasing age of samples from donor CB003



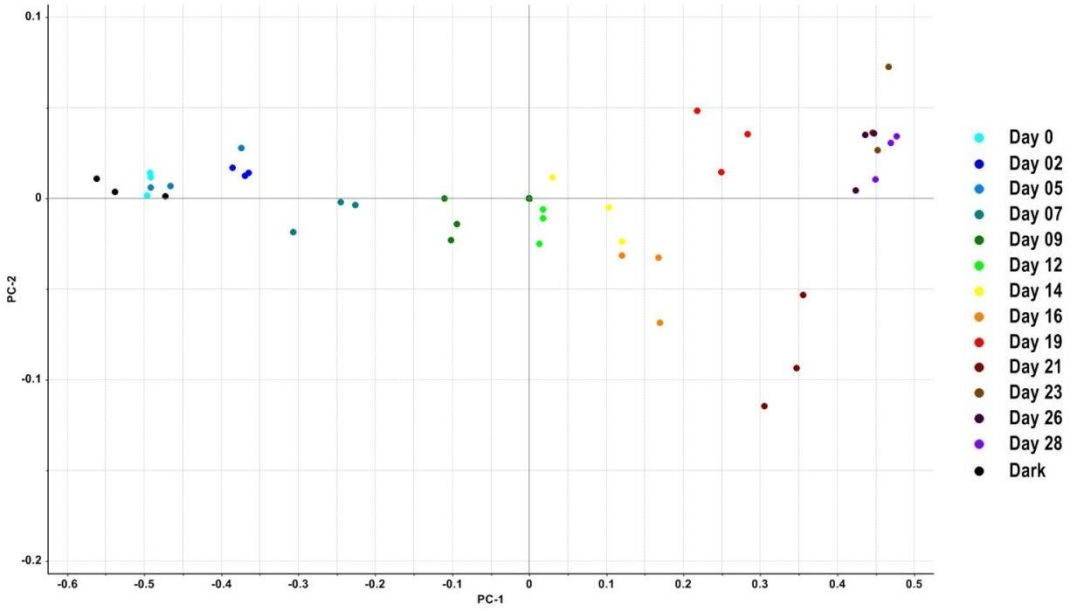
Appendix 3.3: Factor loadings for the first 2 PCs of samples from donor CB003



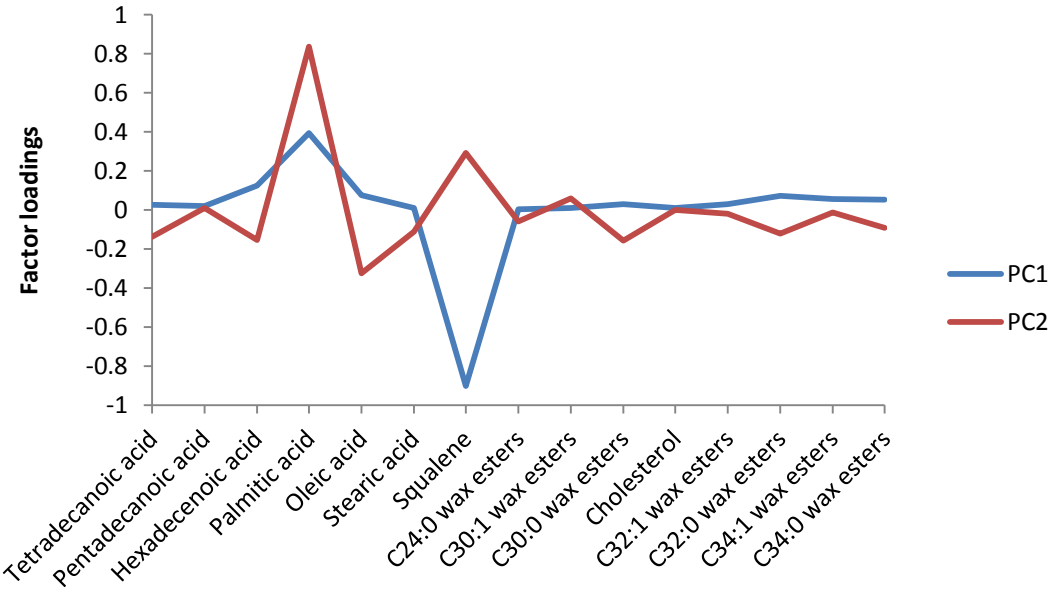
Appendix 3.4: Distance plot constructed from scores of first two PCs, depicting rate of total compositional change over time of samples from donor CB003



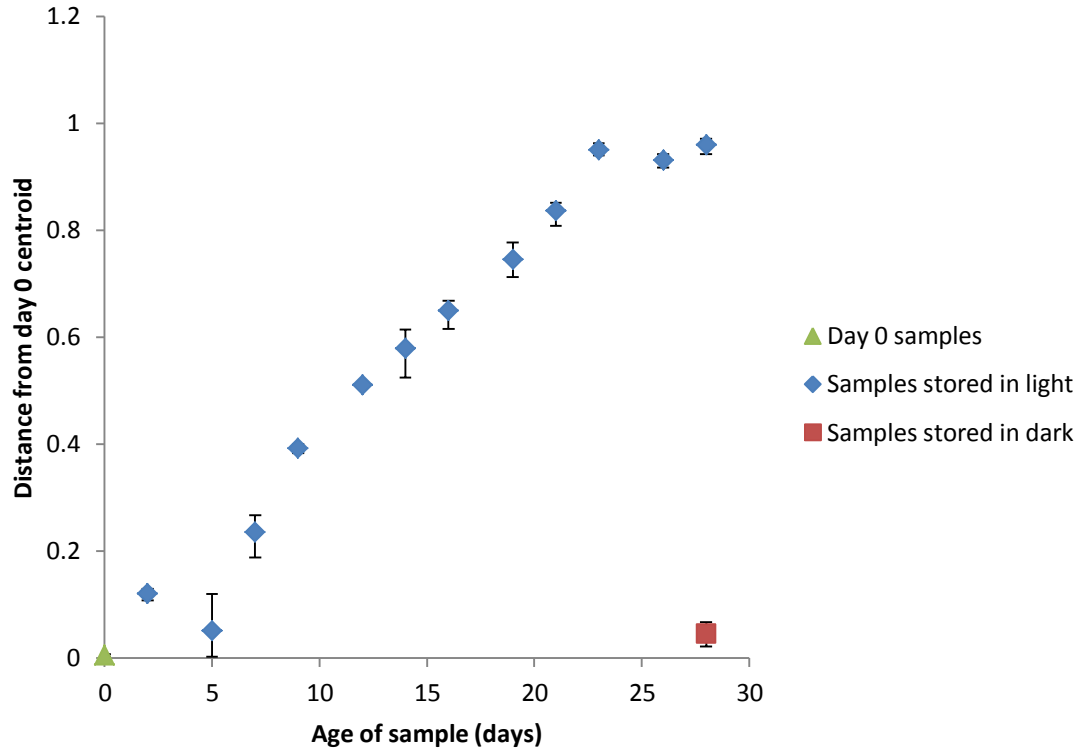
Appendix 3.5: Scree plot depicting the variance in samples from donor CB033 accounted for by each PC



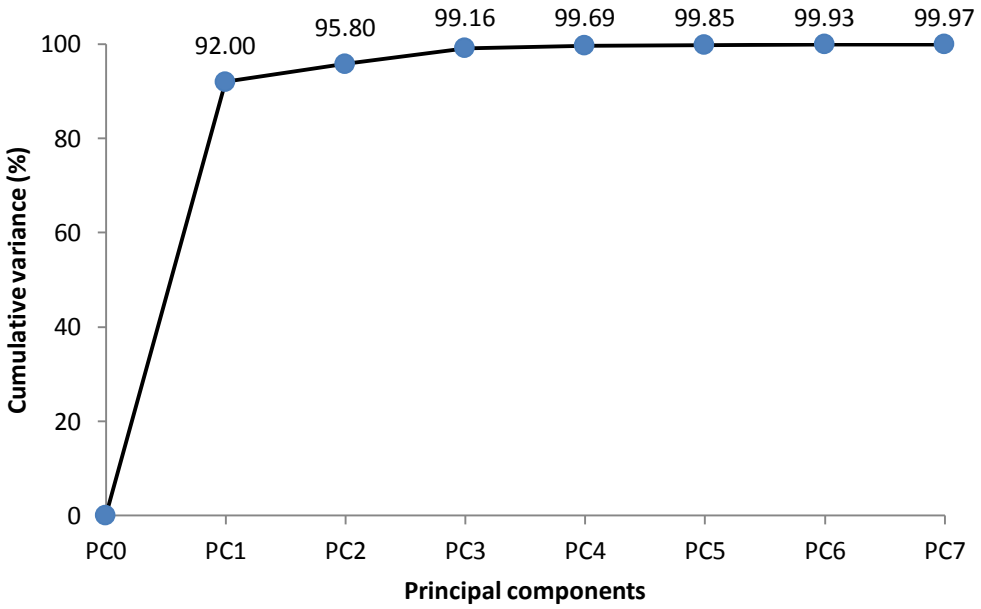
Appendix 3.6: 2-dimensional scores plot generated from the first 2 PCs, demonstrating the distribution of fingermarks of increasing age of samples from donor CB033



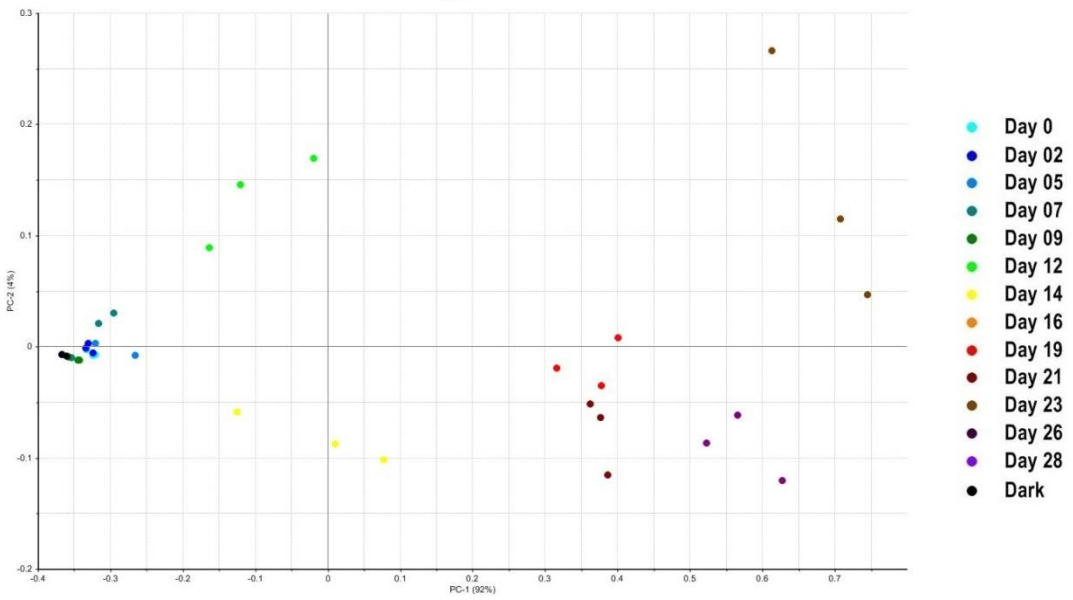
Appendix 3.7: Factor loadings for the first 2 PCs of samples from donor CB033



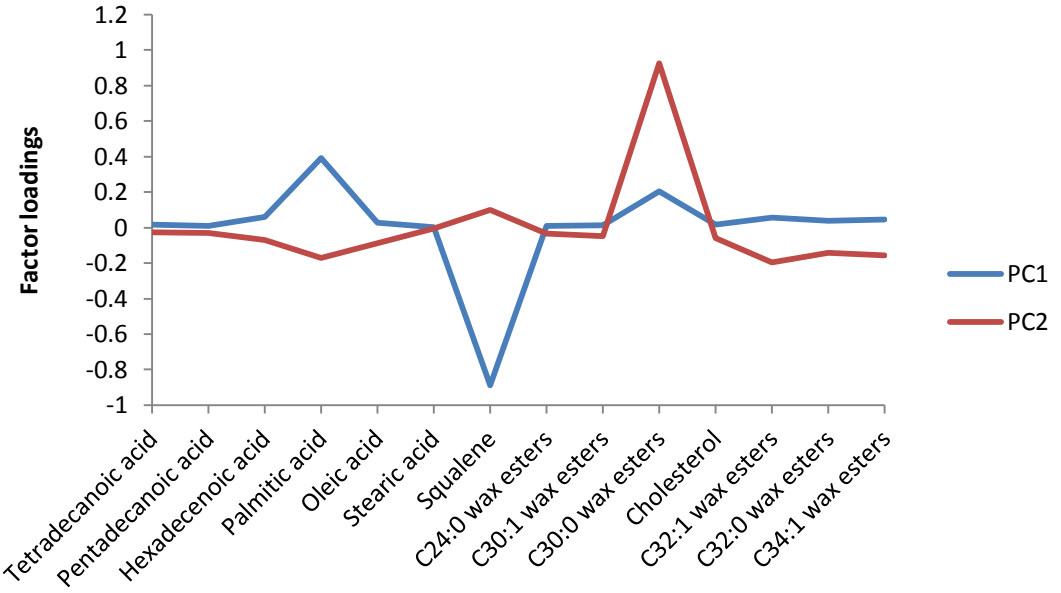
Appendix 3.8: Distance plot constructed from scores of first two PCs, depicting rate of total compositional change over time of samples from donor CB033



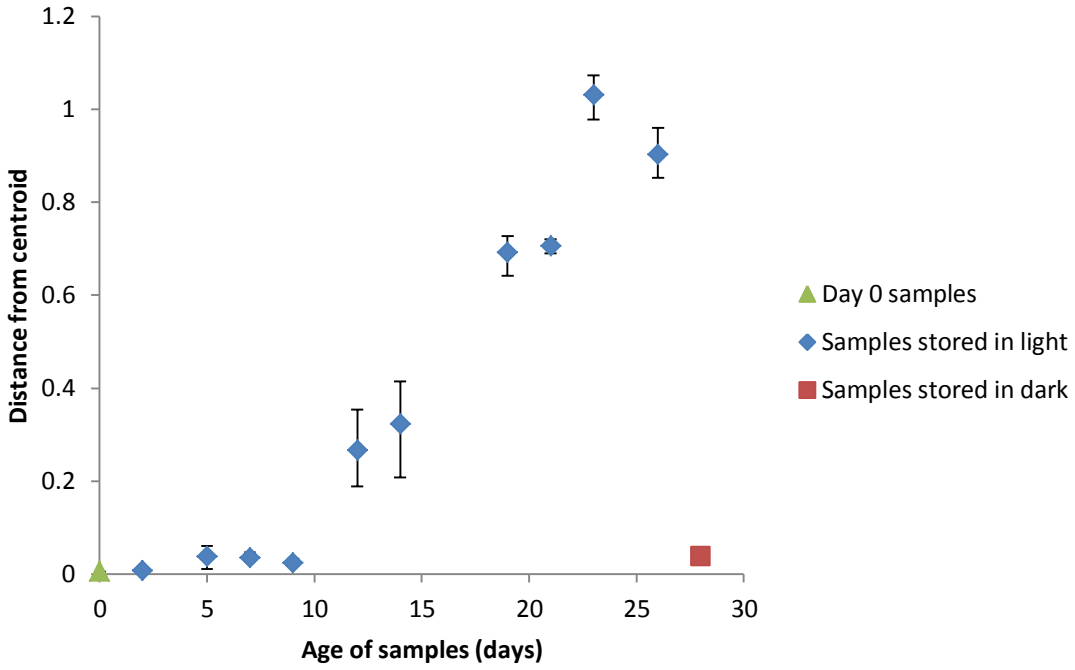
Appendix 3.9: Scree plot depicting the variance in samples from donor CB050 accounted for by each PC



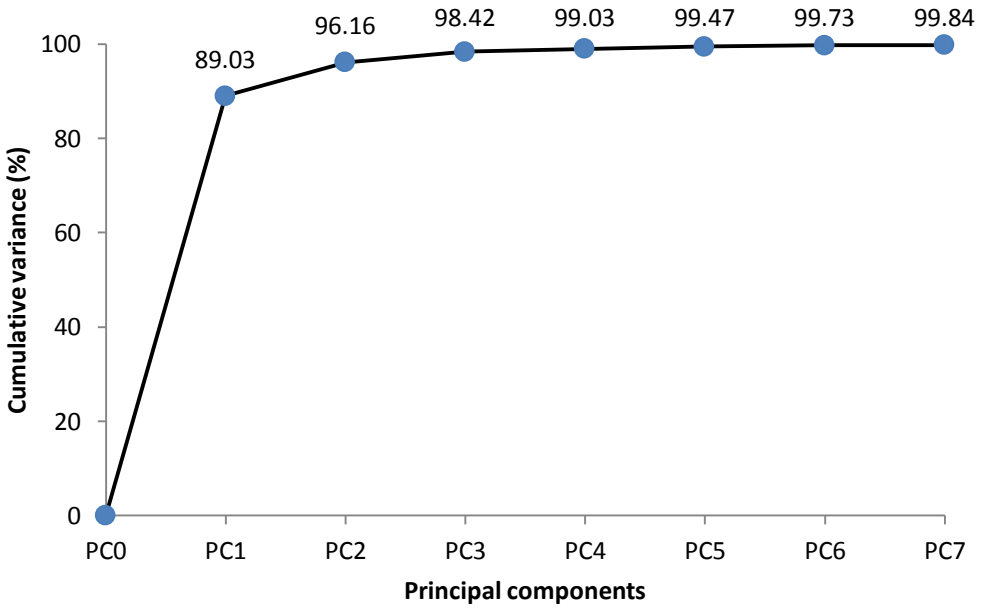
Appendix 3.10: 2-dimensional scores plot generated from the first 2 PCs, demonstrating the distribution of fingermarks of increasing age of samples from donor CB050



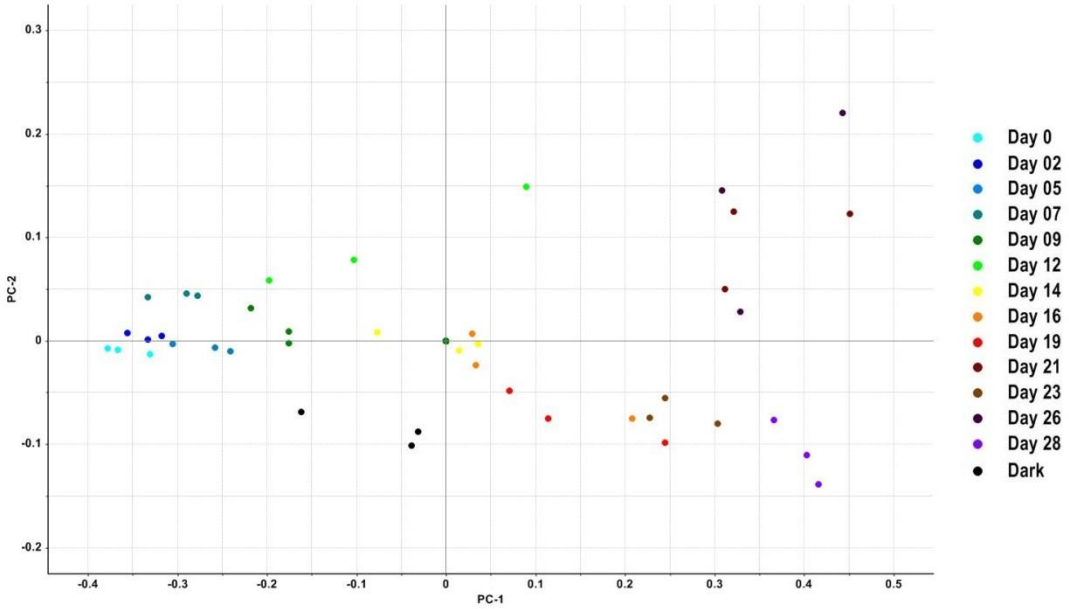
Appendix 3.11: Factor loadings for the first 2 PCs of samples from donor CB050



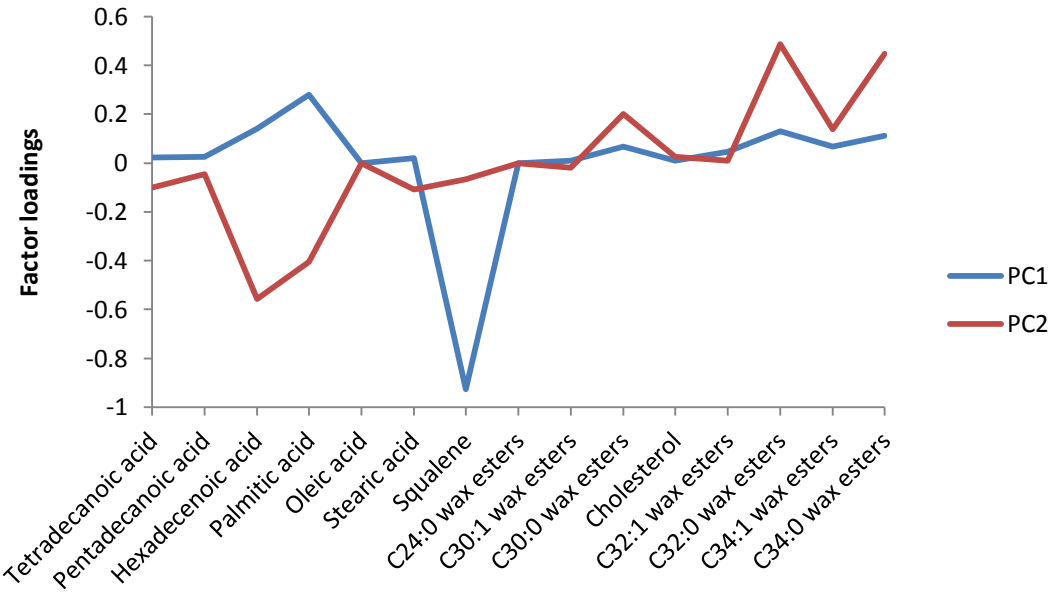
Appendix 3.12: Distance plot constructed from scores of first two PCs, depicting rate of total compositional change over time of samples from donor CB050



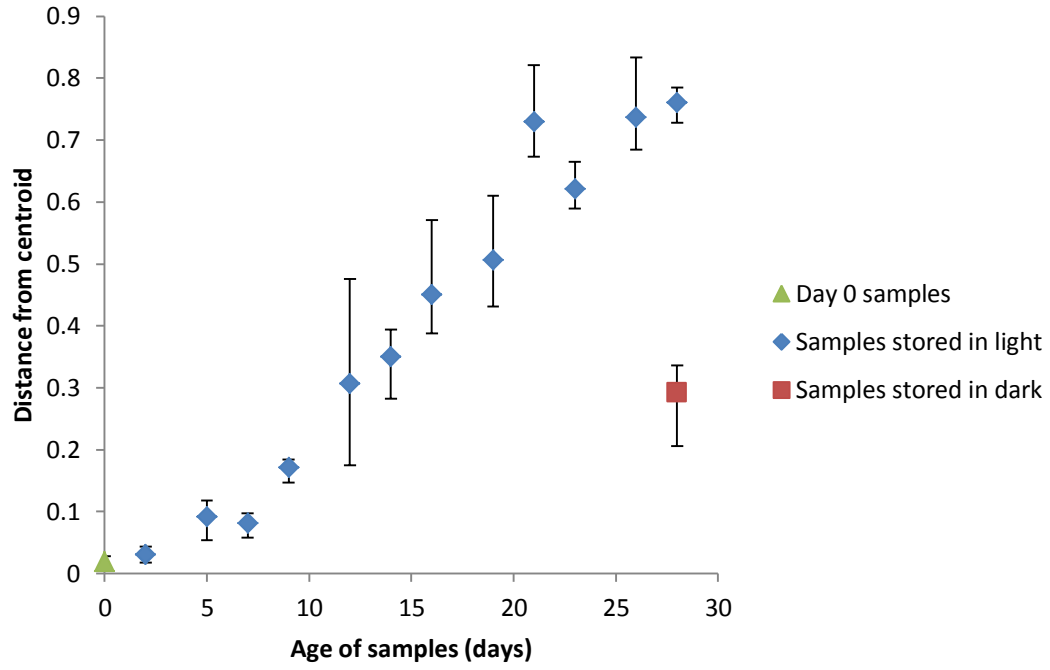
Appendix 3.13: Scree plot depicting the variance in samples from donor DA080 accounted for by each PC



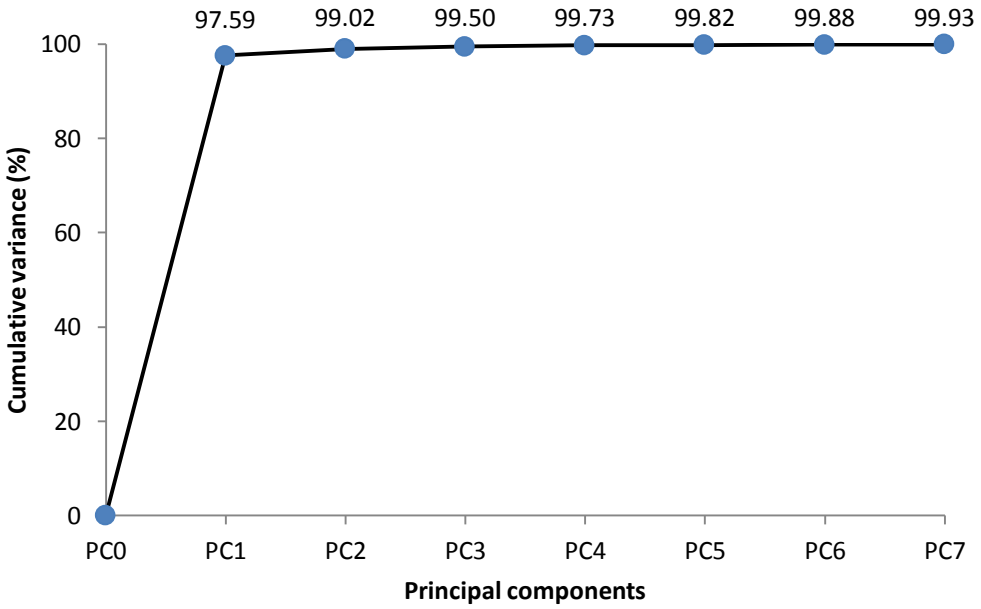
Appendix 3.14: 2-dimensional scores plot generated from the first 2 PCs, demonstrating the distribution of fingermarks of increasing age of samples from donor DA080



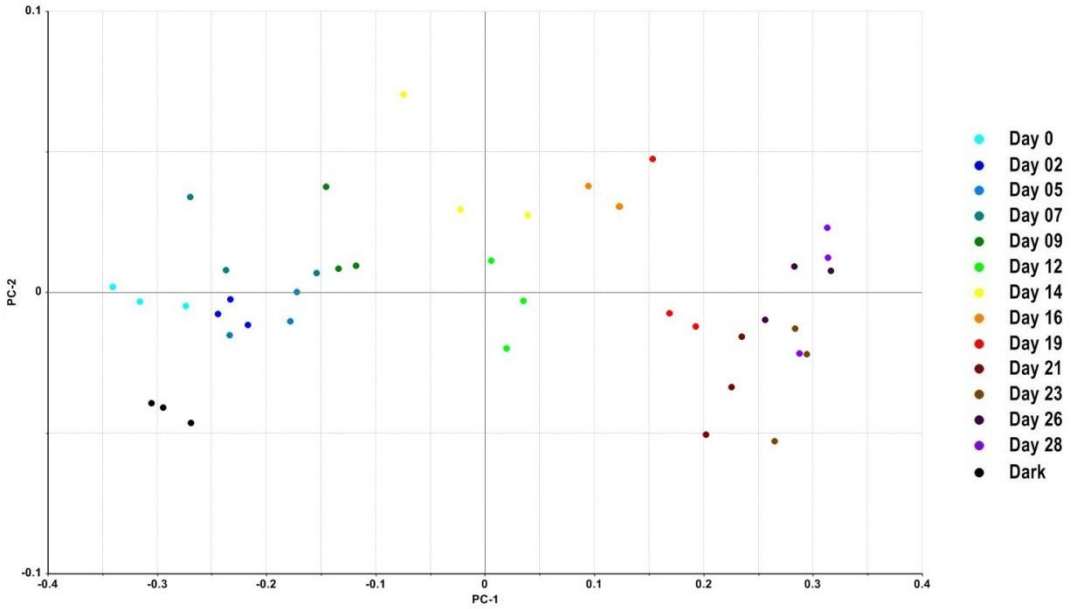
Appendix 3.15: Factor loadings for the first 2 PCs of samples from donor DA080



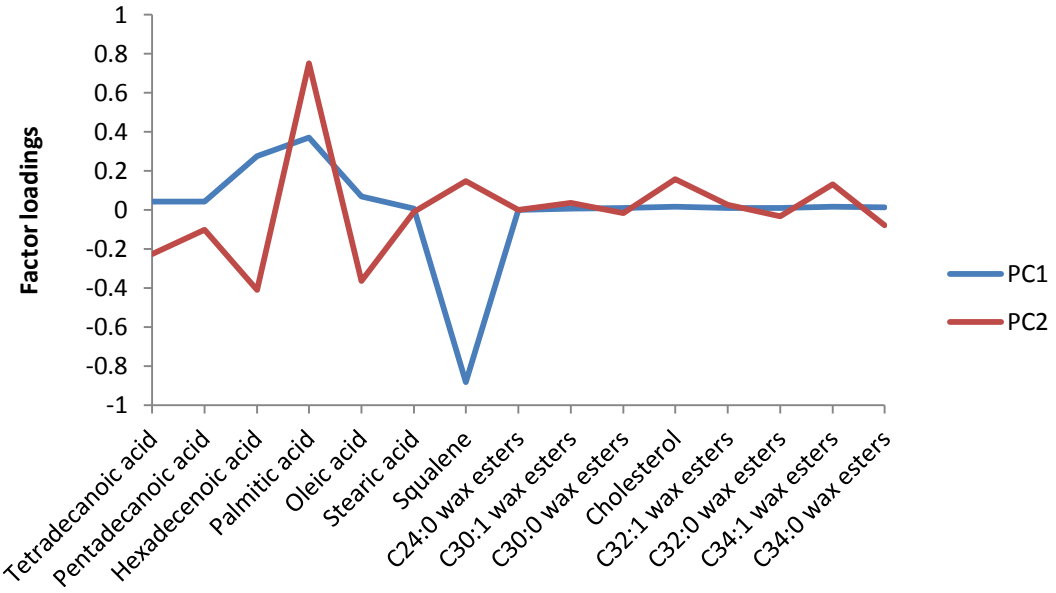
Appendix 3.16: Distance plot constructed from scores of first two PCs, depicting rate of total compositional change over time of samples from donor DA080



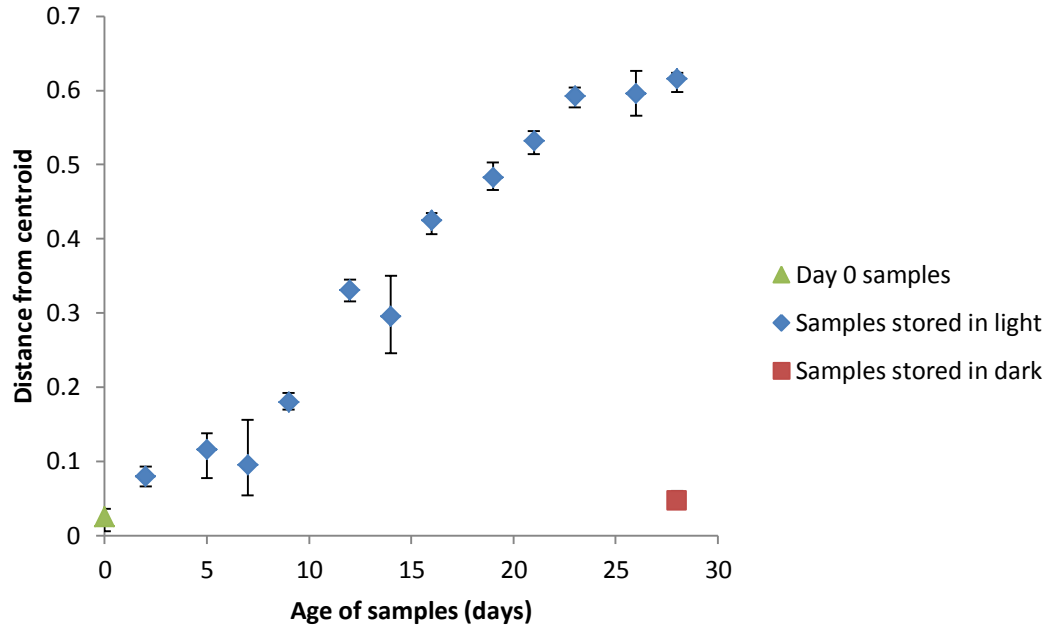
Appendix 3.17: Scree plot depicting the variance in samples from donor CA100 accounted for by each PC



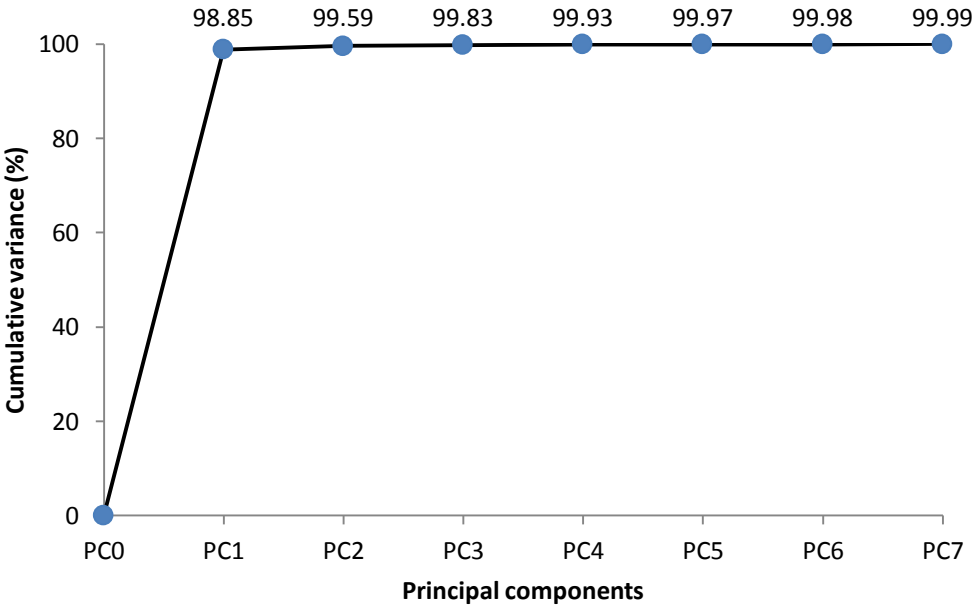
Appendix 3.18: 2-dimensional scores plot generated from the first 2 PCs, demonstrating the distribution of fingermarks of increasing age of samples from donor CA100



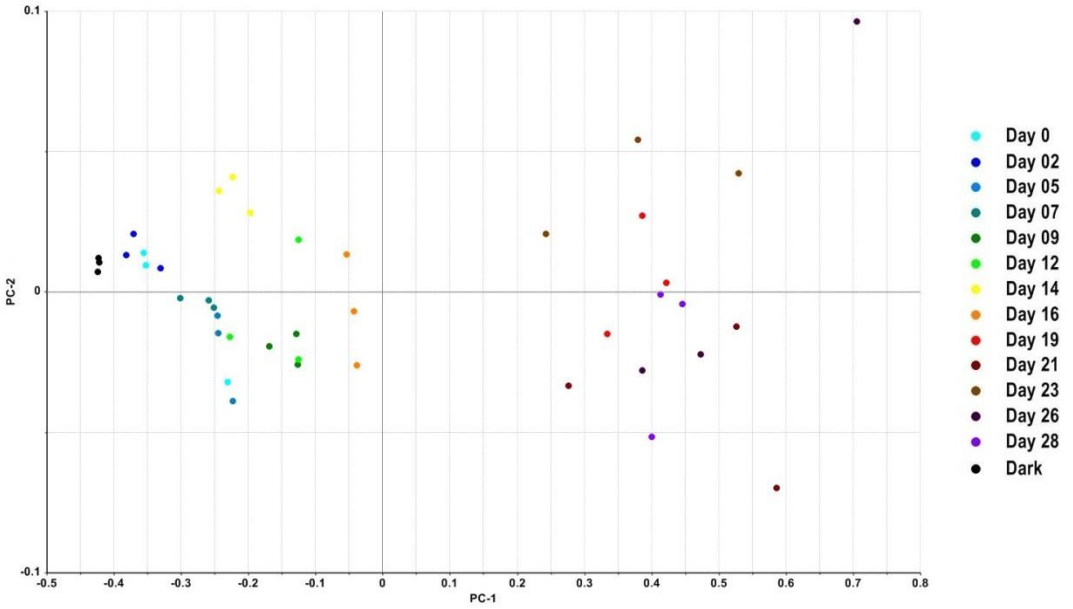
Appendix 3.19: Factor loadings for the first 2 PCs of samples from donor CA100



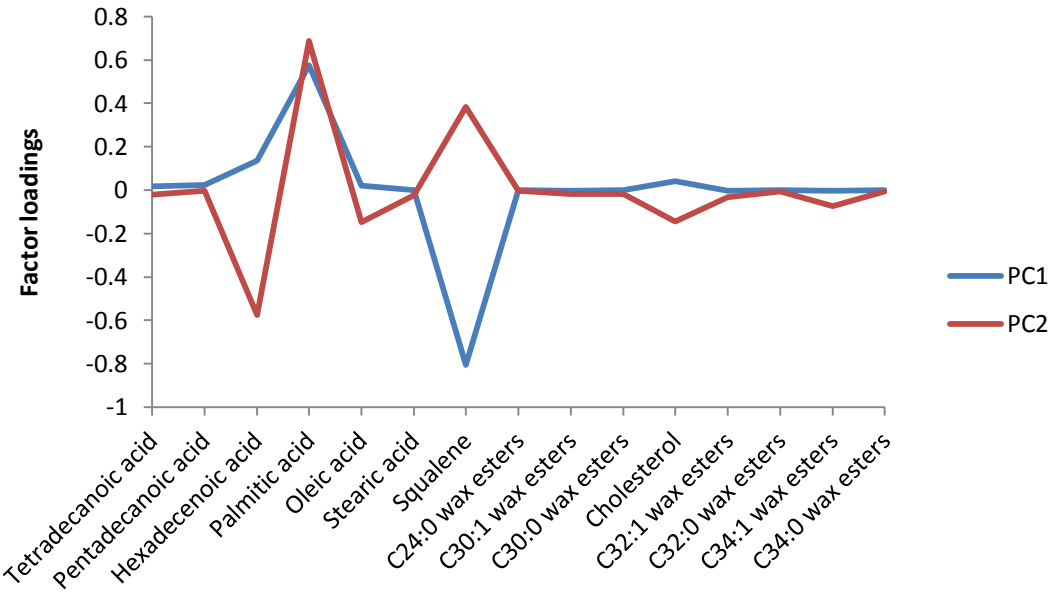
Appendix 3.20: Distance plot constructed from scores of first two PCs, depicting rate of total compositional change over time of samples from donor CA100



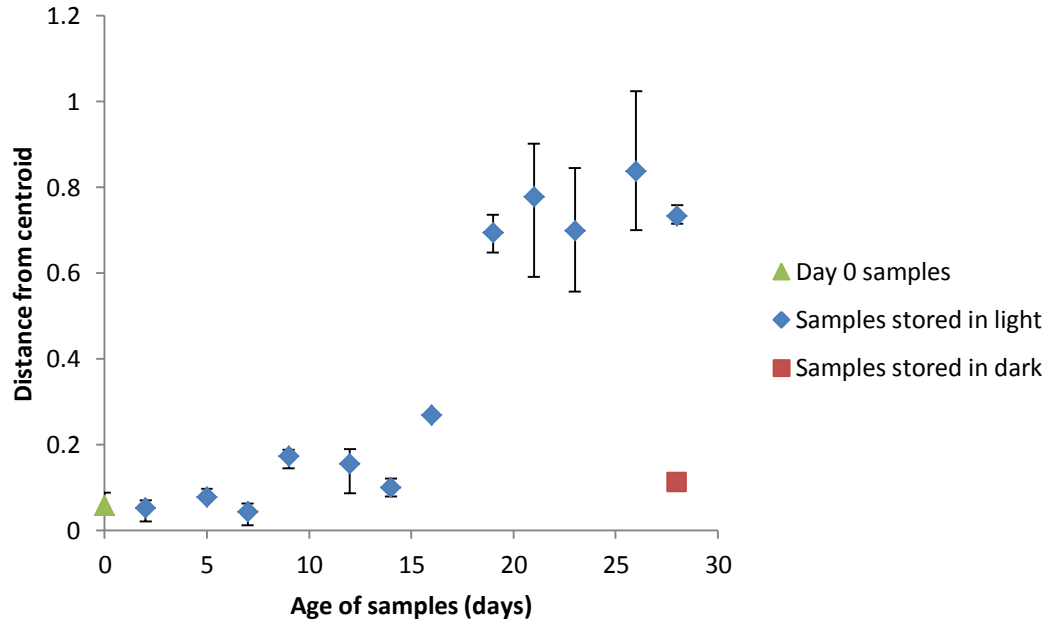
Appendix 3.21: Scree plot depicting the variance in samples from donor DA103 accounted for by each PC



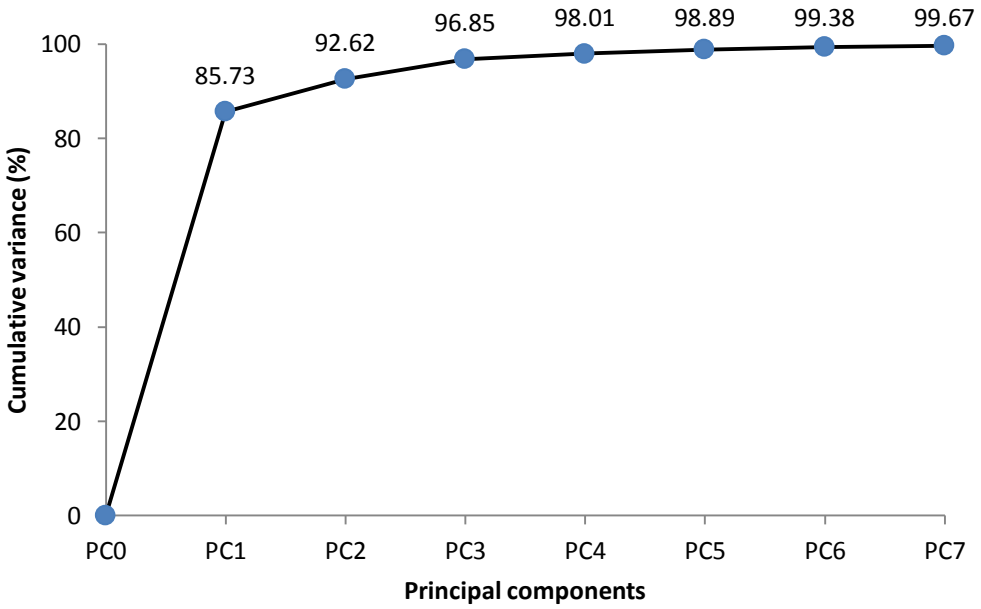
Appendix 3.22: 2-dimensional scores plot generated from the first 2 PCs, demonstrating the distribution of fingermarks of increasing age of samples from donor DA103



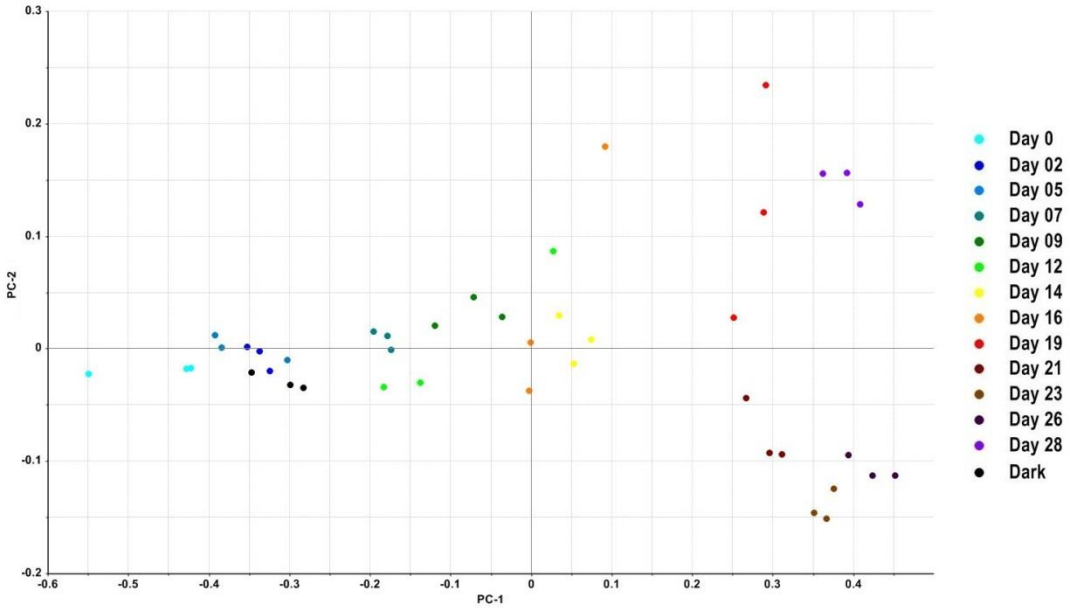
Appendix 3.23: Factor loadings for the first 2 PCs of samples from donor DA103



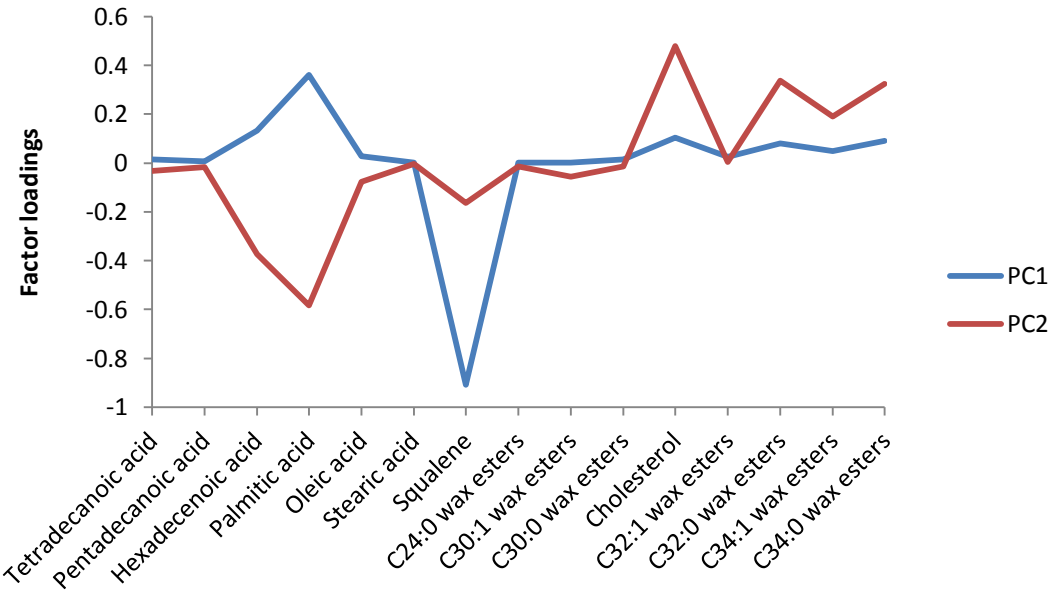
Appendix 3.24: Distance plot constructed from scores of first two PCs, depicting rate of total compositional change over time of samples from donor DA103



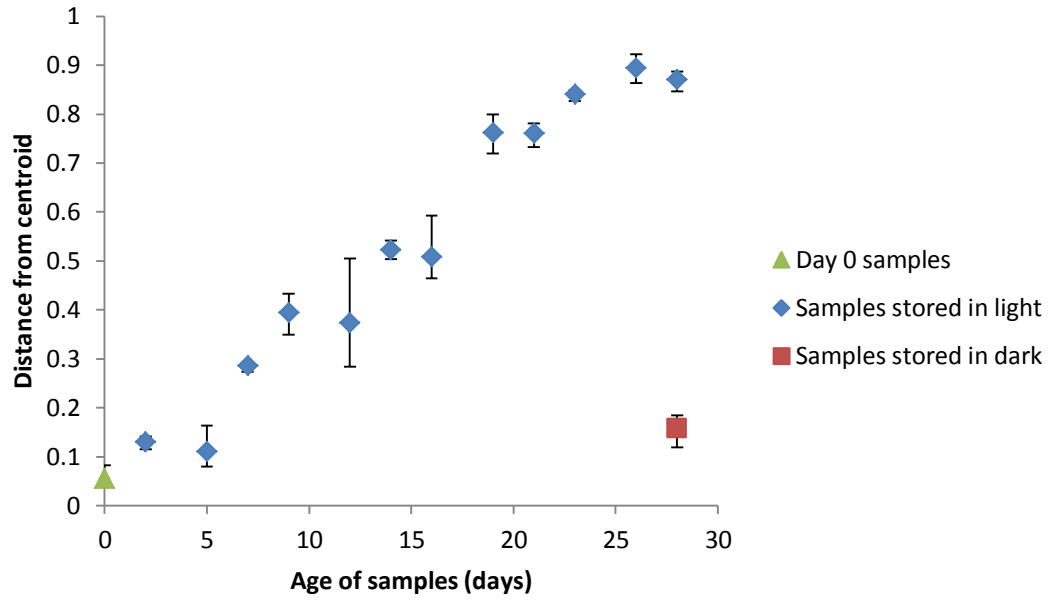
Appendix 3.25: Scree plot depicting the variance in samples from donor CB135 accounted for by each PC



Appendix 3.26: 2-dimensional scores plot generated from the first 2 PCs, demonstrating the distribution of fingermarks of increasing age of samples from donor CB135



Appendix 3.27: Factor loadings for the first 2 PCs of samples from donor CB135



Appendix 3.28: Distance plot constructed from scores of first two PCs, depicting rate of total compositional change over time of samples from donor CB135