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Main Compounds and Major Methods in Latent Fingermark Aging Analysis: a Short Review

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Fingermarks are a complex biological matrix with variability factors that change in the same donor, depending on the moment of collection, and in different donors, according to age, sex and routine. Thus, they can still undergo alterations related to the deposition surface, the environment, and the variable that connects all those mentioned: the action of time. In Forensic Science, time is a important variable to situate the crime events. This review proposes a classification in the temporal estimation research of fingermarks, dividing them into Temporal Preservation Analysis (TPA) and Temporal Aging Analysis (TAA). In TPA studies, the components in fingermark residues undergo a few changes over time, tending to stability after a certain period. Those are interesting targets to identify possible exogenous components, such as firearm residues, illegal substances and contaminants related to particularity of forensic cases. In TAA studies, a time estimation related to the fingermarks age can be established. In this case, the time elapsed from its deposition until the forensic processing will vary according to the component classes degradation. Endogenous and exogenous substances that are demonstrably present in one donor and that undergo changes over time will, resulting be demonstrated by a decrease in intensity and/or formation of other substances, and those are good targets for this type of study. The same analysis can have both proposals and the instrumental method available will enable the extraction of information relevant to the sample. The purpose of this study is to demonstrate how important is to identify fingermarks components as evidence beyond the ridge pattern and to list the main instrumental methods used in the analysis of fingermark degradation.

Keywords: forensic sciences; forensic identification; aging fingerprints; prints residue.

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Introduction

The analytical study of fingermarks is extremely complex. The factors that affect the samples introduce significant variability, which is also influenced by time. To study the composition of fingermarks for the purpose of estimating their age using a specific method, it is crucial to first comprehend the nature of the components comprising this matrix (1).

The biological component found in fingermarks consists of a combination of substances derived from various sources: (a) the epidermis, (b) dermal secretory glands, and (c) extrinsic contaminants (2). Within this framework, eccrine glands, apocrine glands, and sebaceous glands secrete substances that interact with intrinsic epidermal components, including metabolites and traces of drugs, as well as extrinsic contaminants such as dirt, grease, dermocosmetics, and other pollutants (2).

In addition to the biochemical degradation of the components described above, there are factors that influence this degradation, which can either accelerate or slow down the process. These factors include the initial composition of the fingermark, the deposition substrate, the environment, and time (3).

Time is a universal variable that plays a crucial role in fingermark detection studies, including fingermark aging studies. It affects different components of fingermarks to varying degrees (4). Building upon this understanding, a review was conducted proposing a classification of temporal studies based on two types of components.

The first type involves components that undergo alterations over time but still retain the potential for detecting fingermarks with distinguishable ridges even after a certain period has elapsed. These components exhibit relative degradation but tend to reach a stable state over time. The second type comprises components that undergo degradation, progressing through reaction intermediates until they are – almost – completely extinct. As a result, their detection becomes challenging or impossible due to the passage of time.

This classification can help to differentiate between components that exhibit varying levels of stability and degradation in fingermarks as time elapses.

Many research groups have dedicated themselves to the study of fingerprint degradation. However, due to the variable nature of the matrix, there is still no accepted and validated protocol for use in Forensic Science. Therefore, the idea of separating aging studies aims to facilitate the direction of research towards the detection of fingerprints after a certain period has elapsed or the characterization of their degradation.

Thus, the objective of this study is not to separate or segregate samples, methods, or research types based on the components. Instead, the goal is to emphasize the significance of different classes of components within fingermark samples and how they collectively contribute to elevating this biological matrix as a valuable source of chemical intelligence.

Experimental section

This review was conducted by researching articles from two databases: Science Direct and Google Scholar. The selected articles cover the period from 2013 to 2023 and include review articles and data articles published in English that utilize instrumental methods for the analysis of fingerprint aging. Figure 1 illustrates the selection process and the division of articles by instrumental classes.

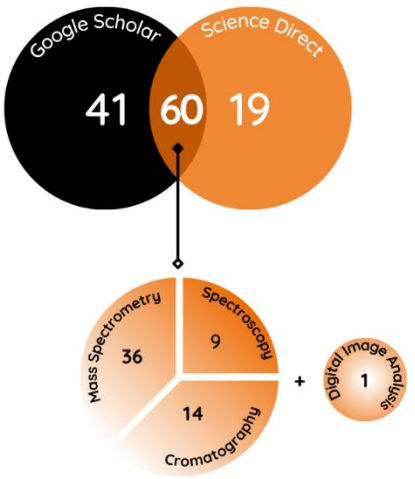

Figure 1. Chosen databases, number of selected articles and instrumental methods used for fingermark ageing analysis.

Results and Discussion

A total of 60 articles were selected, prioritizing those that used instrumental methods to analyze fingerprint degradation. The instrumental class with the highest number of results is mass spectrometry (5 – 36). Generally, this class of techniques is highly sensitive, but data analysis is complex. Studies involving the use of chromatography (37 – 46) are mainly focused on the analysis of sebaceous components. On the other hand, spectroscopy methods (47 – 55) involve studies on chemical profiling, differentiation between donors, and degradation of components over time. Among the three classes of methods, spectroscopy has the lowest analysis cost, requires minimal sample preparation, provides results in seconds, and can utilize multivariate tools for result analysis, enhancing the technique. In addition to traditional techniques, two studies that make use of digital images and statistical tools were selected, which can be a good alternative for preliminary tests or in forensic laboratories with limited resources.

Fingerprints are the most common traces left at a crime scene. Temporality is a recurring issue in different investigation circumstances, as suspects may claim that they left the traces at a different time than the occurrence of the crime. Generally, the lack of protocols and validated methodologies accepted by the forensic community elevates the importance of research in this matter (9).

Degradation research has a prevalence due to the importance of time in forensic cases. After all, the understanding of placing events in time indicates the improvement of forensic procedures. In addition, the search for detection of components present in fingermarks considers this sample capable of identifying certain elements that may be important in crime investigation (1, 9).

Understanding the environment and time variations that act in the of fingermarks detection, as well as identifying stable products that act as targets in the detection of components are some strategies to model and plan a degradation study with these samples towards a reliable standard (1 – 3).

Thus, the results were compiled by the class of endogenous (eccrine and sebaceous) and exogenous (external contaminants) components to better understand the studies that explore the time variable. The classification of the samples was performed in two ways: (1) Temporal Aging Analysis (TAA) for components that suffer degradation to the point of not being identified by the proposed methods, being targets for fingermark aging estimation, and (2) Temporal Preservation Analysis (TPA) for components that suffer the action of some other variable that delays their degradation or that are less reactive species by nature, being considered appropriate targets to detect a fingermark after a time of deposition.

Endogenous Compounds

1. Eccrine Compounds

Eccrine sweat is important in the fingermark components research, as it is the content secreted from the fingers, but it presents a lot of variability in detection and tends to lose a large amount of the material present after a short period. It is the primarily agents that suffers the effects of degradation and it is responsible for the significant losses

of material present in the fingermarks in the first 48 hours (56).

The main representative of eccrine sweatis water, which is not a good parameter to evaluate TAA or TPA, as it undergoes almost immediate volatilization after deposition and which lasts for an uncertain period within days, according to other variables such as ambient temperature, surface and humidity. It has been suggested that, if water is present in a fingermark if (demonstrated, for example, by H-O broadening in the region of 3000 in an infrared spectrum), it can be concluded that the fingermarks are freshly deposited (52, 56). Overall, 99% of eccrine sweat is constituted of water, but this percentage varies from 20 to 70% depending on the sample donor, consideringother constituents and even whether the sample donor washed their hands or not. Amino acids, peptides and proteins, in general, can be considered TAA and TPA, but the age estimation for these components is not clear in the studies. Some researchers argue that the the concentration of nitrogenous components varies in fingermark samples. Still, these components can also be degraded by bacteria (2, 3, 18, 22). As large organic molecules, they can undergo transformation over time, but they tend to have a stability that can be detected in months or years. Ninhydrin, a latent fingermark developer, is mainly applied to recover latent fingermarks on smooth surfaces such as paper, and it is capable of detecting protein components that are trapped in the cellulose fibers. Thus, cellulose acts as a factor that interferes the degradation of fingermarks. Frick *et al.* (2021) detected proteins in fingermarks after 236 days of deposition, but without emphasizing the mechanisms behind it (3). Protein components are susceptible to thermolabile variations that can completely degrade their constituents. However, its been shown in the literature that degradation products can also be identified. For example, tryptophan forms a complex with proteins that result in auto fluorescent compounds such as indoleacetic acid, making it possible to estimate the age of the sample. In a proteomic study, Oonk et al. measured an estimate of 16 days for dermicin, while some keratins generate fewer stable products, not being detected throughout the evaluated period (54). Other components such as lactic acid, which is the target of cyanoacrylate fumigation, undergo photooxidation to pyruvate at 50 ºC, which makes it difficult for the cyanoacrylate to adhere, suggesting that samples in which adherence does not occur so well have already undergone lactic acid degradation (28).

2. Sebaceous Compounds

Sebaceous components are present in fingermarks samples through contamination by touching oily regions of the body, such as face and ears (34,45). These components play an important role in the fingermarks detection in situations where the sample has been wet or when the eccrine components have already degraded. In general, they fit as both TAA and TPA. Sebum present in humans is rich in unsaturated lipids and undergoes successive modifications in reaction intermediates, being attractive for aging studies. It is also largely responsible for the variability of intra and inter donor fingermarks samples, due to widespread contamination (3, 5, 8, 14, 15). Over time, it may present viscosity changes, becoming more solid due to progressive oxidation, making the use of some dyes and developers difficult.

Girod et al. (2016) detected more free fatty acids in over one month of analysis by GC-MS, with the components undergoing degradation (36). Pleik et al. had similar results evaluating14 days of degradation. This can classify free fatty acids into TAA. Oleic acid by ozonolysis forms reaction intermediates that can be detected over time, also fitting the TAA classification (19, 55). The ozonolysis reaction appears to be important in the aging process because it occurs in many unsaturated triglycerides. From this reaction, mono- and di-ozonide intermediates arise and are detected in fingermarks residues. It is evident that, over time, the unsaturated lipid fraction undergoes changes due to degradation to become increasingly saturated, depleting glycerides and free fatty acids by the ozonolysis reaction (55, 58). These changes occur shortly after the fingermarks is deposited and last for unclear periods. Thus, when some components undergo successive oxidations, it can be classified as TAA and, when generating a stable final product (waxy and viscous), it can be classified as TPA.

Squalene and cholesterol need special considerations. Squalene molecule has many unsaturation bonds and is considered the most reactive surface lipid on the skin surface (55). In contrast, cholesterol is more stable. However, both undergo similar degradation processes by singlet oxygen, ozonolysis, and UV light. Squalene degradation is one of the most reported in fingermarks aging estimation studies, resulting in volatile intermediates that can be detected up to one month of analysis (according to what is reported in the literature until now) (35 – 38), being classified as TAA. After the oxidation of squalene to short-chain degradation products that can be detected 5 to 10 minutes after deposition in a chromatographic method (GC-MS) as epoxides and hydroperoxides that are already formed soon after the deposition of the fingermarks (36). The oxidation reactions continue to occur in second and third intermediates that form ketones and aldehydes (volatile compounds). In a study using the methodology of UHPLC-HRAM OrbitrapTM MS showed that the degradation reactions occur in the skin, even before the deposition of the fingermarks on surface. Thus, it was possible to see that the oxidation reactions occurred previously and simultaneously during aging (35).

Cholesterol and its sterol esters are on average present in almost 5% of human sebum and there is little research on their degradation. However, its stability reported by Weyermann et al. (2021) may give it a relevant component for TPA studies. Wax esters are present in fresh and aged fingermarks, however there is no clarity on the mechanisms that lead to the degradation of these components, it is suggested that their degradation occurs more slowly, being a potential TPA (3, 37, 40, 50). In general, in lipid and glycid components, when it has an

unsaturated chain, it undergoes ozonolysis and other reactions in order to become stable or saturated. Thus, it can be classified as TAA. The lipid and glycid components of the saturated chain are almost inert and can be classified as TPA (41).

Exogenous Compounds

The main exogenous compounds found in fingermarks are often linked to the type of crime being investigated. Drugs, explosives, firearm residue, and condom lubricants illustrate this category (4,17).

Exogenous compounds are residues that come from the individuals' hands after touching different substances, surfaces, or food, but also other parts of the body. When evaluating the degradation of sebaceous components, it is common to ask the donor to run his fingers over his forehead, because the sebaceous components are still a contamination.The palms of the hands and soles of the feet do not secrete sebaceous components, only eccrine compounds (25,52).

Soaps, creams, makeup, essential oils and dermocosmetics are described as important contaminants that interfere in many analyses. In mass spectrometry, for example, the dimethyldioctadecylammonium signal, *m/z* 550.6, a surfactant found in different types of soap, has high intensity, which may hinder the detection of endogenous components (4,7,11,17,44).

The analysis of exogenous components of fingerprints can be classified mainly as TPA, since many of these components are stable or their degradation products are easily detected. When using the fingermark as evidence of the presence and/or authorship of a crime, the specificity of the contaminant can place the suspect at the crime scene.

3. External Contaminants

Analytical Methods for Components Detection

The selection of an analytical method needs to take into consideration the nature of the sample and its availability, whether it requires analyte extraction, and what analytical problem it aims to solve with the technique's application. Considering fingermarks as a biological sample, it is known that the amount of material available for analysis is small and highly susceptible to contamination. Therefore, the choice of an instrumental method that does not require elaborate sample preparation becomes not only advantageous but essential (1,2)

Approaches for using instrumental methods for fingermark dating research involves: i) investigative information (such as the surface of deposition and the maximum interval between deposition and discovery of the fingermark); ii) timeline of deposition (in case of fingermark overlap, determining if they are from the same or different donors and which one was deposited first), and iii) physical and chemical changes of the sample over time (details like degradation pathways and visible degradation of the fingermark ridges). The third point is the main topic of discussion here (40).

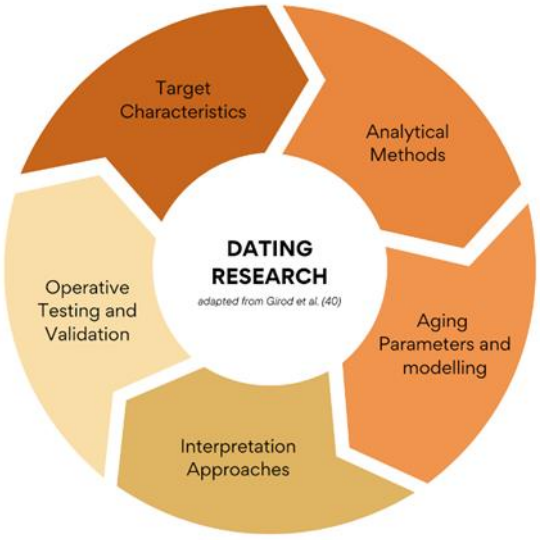

Figure 2. Proposed approach to address the fingermark dating issue considering the research.

Two considerations that cannot be overlooked are the available resources for conducting the analyses, meaning a technique with broad applicability would also have an advantage in this scenario. Additionally, concerns about the environment, both in terms of waste generation and analyst occupational safety, should be taken into account. Therefore, methods that allow for direct analysis of the samples should be considered (40, 60).

Girod et al. (2016) proposed a research cycle to develop and test analytical methods for fingermark degradation analysis, adapted in the following Figure 2 (40).

Fourier Transform Infrared Spectroscopy (FTIR) is one of the main techniques used to characterize the composition of fingerprints, specifically lipid components (44 - 51), and the amino acid serine has also been the subject of study (51). The technique allows for differentiation between donors of the same sex over time, as demonstrated in González *et al*. (2021). The advantages of using Infrared (IR) are direct analysis, non-destructive to the fingerprint ridges, allows material collection for DNA examination, rapid analysis, and does not require the use of reagents. However, the limiting steps of IR studies are that they are semi-quantitative and do not work on all surfaces where the fingerprint was deposited. (45-47).

Raman Spectroscopy has been a recent alternative, used to identify, mainly exogenous components **(**contaminants) such as drugs, medications and explosives, but it has also been applied for nonspecific detection of eccrine (protein) and sebaceous secretions (48). Some signals were attributed to squalene and fatty acids and showed a decrease in relative intensity after one month of analysis, however the variability was also discussed and the results

need to be interpreted cautiously through replicated analyzes enough to be sure of what is detected (49, 53).

Many methods are more invasive than optical methods, but are more specific, quantitative and reproducible, having fundamental research potential despite being more expensive, time consuming and complex to apply. This is the case of Gas and Liquid Chromatography separation techniques, commonly coupled to a mass detector. Gas Chromatography Coupled Mass Spectrometry (GC-MS) usually targets volatile and thermally stable compounds with small amino acids and lipids. While Ultra-Performance Liquid Chromatography coupled to Mass Spectrometry (UPLC-MS) is suitable for larger molecules such as proteins, peptides and glycerides, and may help in the identification of substances that work as target compounds for monitoring the aging of fingermarks (43).

Several alternative methods of mass spectrometry have also been used to study fingerprint chemical composition and, more specifically, chemical imaging of endogenous and exogenous compounds (4 – 35). The Matrix Assisted Laser Desorption/Ionization (MALDI) method is well developed by the group of Francese and collaborators for analysis of exogenous components, superimposed fingerprints, chemical imaging to study the diffusion of saturated fatty acids over time (5, 14, 29, 32).

The use of digital images for forensic applications is already a reality in facial recognition, in identification of Cannabis sativa L. plantations by drone images, and recently, a study proposed by De Alcaraz-Fossul *et al.* (2020) uses the mean intensity and range of color intensity from the digital photographic record of fingermarks developed with powders with statistical application to evaluate temporal estimation (60). The use of statistical methods combined with imaging tools can be applied as a way of screening samples for later use of more robust techniques.

In general, spectroscopy, mass spectrometry and chromatography techniques can be applied to eccrine, sebaceous and exogenous compounds. The selection of the technique will depend on the objective of the study: that of evaluating the time or identifying a suspect, considering the temporal estimate of the deposition of the suspect's impression in the place of interest. Once the purpose of the study is known, the target compounds of interest can be selected, such as drugs of abuse, metabolites, sebaceous or eccrine compounds, and then select the most applied techniques and propose an analysis test. The following table selects some target components and the most used methods.

Table 1. Main target compounds for fingermark aging analysis and the respective most used methods.

Targets	Analytical Method
Squalene	FTIR[1] RAMAN[2] GC-MS[3] LC-MS/MS[4] MALDI-MS[5]
Cholesterol	FTIR[1] RAMAN[2] GC-MS[3] LC-MS/MS[4] MALDI-MS[5]
Lipids	FTIR[1] RAMAN[2] GC-MS[3] LC-MS/MS[4] MALDI-MS[5] UPLC-MS[6]
Amino acids	RAMAN[2] GC-MS3
Proteins	LC-MS/MS[4]
Exogenous	FTIR[1] RAMAN[2] GC-MS[3] LC-MS/MS[4] MALDI-MS[5]

[1]Fourier Transform Infrared; [2] Raman Espectroscopy; [3]Gas Cromatography Coupled with Mass Spectrometry; [4]Liquid Cromatography coupled with Mass Spectrometry; [5] Matrix Assisted Laser Desorption/Ionization; [6] Ultra-Performance Liquid Chromatography coupled to Mass Spectrometry

Conclusions

A review about fingermark components and analysis was presented based on the classification of temporality studies of these samples. Endogenous samples can be useful for fingermark aging studies. Although it is impossible to predict a date, it is possible to make an estimate according to the monitoring of the degradation intermediates of some components. As there is considerable variation in triglyceride concentration in human secretions with a high content of unsaturated components, degradation processes can be used to estimate the age of a fingermark, such as squalene. Exogenous components, on the other hand, have a strong classification potential in the preservation of fingermarks over time, which can provide relevant information for investigation intelligence.

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Conflict of interest

The authors declare no conflicts of interest.

Supplementary sections

The table referring to the articles used for this review was attached to the supplementary material.

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