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Lipid composition of fingermark residue and donor classification using GC/MS

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Abstract

Lipids available in fingermark residue represent important targets for enhancement and dating techniques. While it is well known that lipid composition varies among fingermarks of the same donor (intra-variability) and between fingermarks of different donors (inter-variability), the extent of this variability remains uncharacterised. Thus, this worked aimed at studying qualitatively and quantitatively the initial lipid composition of fingermark residue of 25 different donors. Among the 104 detected lipids, 43 were reported for the first time in the literature. Furthermore, palmitic acid, squalene, cholesterol, myristyl myristate and myristyl myristoleate were quantified and their correlation within fingermark residue was highlighted. Ten compounds were then selected and further studied as potential targets for dating or enhancement techniques. It was shown that their relative standard deviation was significantly lower for the intra-variability than for the inter-variability. Moreover, the use of data pre-treatments could significantly reduce this variability. Based on these observations, an objective donor classification model was proposed. Hierarchical cluster analysis was conducted on the pre-treated data and the fingermarks of the 25 donors were classified into two main groups, corresponding to "poor" and "rich" lipid donors. The robustness of this classification was tested using fingermark replicates of selected donors. 86% of these replicates were correctly classified, showing the potential of such a donor classification model for research purposes in order to select representative donors based on compounds of interest.

Keywords: fingerprints, sebaceous compounds, variability, chemometrics, hierarchical cluster analysis

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Abstract

Lipids available in fingermark residue represent important targets for enhancement and dating techniques. While it is well known that lipid composition varies among fingermarks of the same donor (intra-variability) and between fingermarks of different donors (inter-variability), the extent of this variability remains uncharacterised. Thus, this worked aimed at studying qualitatively and quantitatively the initial lipid composition of fingermark residue of 25 different donors. Among the 104 detected lipids, 43 were reported for the first time in the literature. Furthermore, palmitic acid, squalene, cholesterol, myristyl myristate and myristyl myristoleate were quantified and their correlation within fingermark residue was highlighted. Ten compounds were then selected and further studied as potential targets for dating or enhancement techniques. It was shown that their relative standard deviation was significantly lower for the intra-variability than for the inter-variability. Moreover, the use of data pre-treatments could significantly reduce this variability. Based on these observations, an objective donor classification model was proposed. Hierarchical cluster analysis was conducted on the pre-treated data and the fingermarks of the 25 donors were classified into two main groups, corresponding to "poor" and "rich" lipid donors. The robustness of this classification was tested using fingermark replicates of selected donors. 86% of these replicates were correctly classified, showing the potential of such a donor classification model for research purposes in order to select representative donors based on compounds of interest.

Keywords: fingerprints, sebaceous compounds, variability, chemometrics, hierarchical cluster analysis

1. Introduction

During the last 50 years, numerous forensic publications focused on the study of fingermark composition [1-15]. These studies showed that fingermark residue mainly contains eccrine (proteins, amino acids, salts and inorganic compounds) and sebaceous compounds (glycerides, wax esters, fatty acids, squalene, cholesterol and sterol esters). It was also observed that the composition of fingermark residue is highly variable [2-15]. In fact, this composition was defined as a complex system changing over time from an "initial composition" state to an "aged composition" state; the whole system being significantly influenced by numerous factors. These influence factors have been classified in five groups affecting the initial composition and/or the aged composition [1]: (1) donor characteristics, (2) deposition conditions, (3) substrate nature, (4) storage conditions and (5) contaminations. Therefore, it was emphasised that knowledge about fingermark composition is still incomplete. In fact, more qualitative and quantitative data should be acquired concerning the initial composition and an in-depth study of its variability is also necessary. Furthermore, information is also missing about aging kinetics and influence factors affecting the fingermark residue over time [1, 8, 14].

This lack of knowledge can be an issue for the development and/or improvement of enhancement techniques. For example, the target compounds of the physical developer are still not precisely known. Lipids definitively play a role in this reaction, but the research on that issue did not identify which lipids and which reaction pathways were producing the fingermark enhancement [16]. Thus, there is a need for more qualitative data in order to solve this issue. Furthermore, fingermarks of different types of donors are usually chosen based on subjective criteria to conduct representative experiments on enhancement techniques. The donors are thus classified into "poor", "medium" and "good" donors within their institution/research laboratory, based on the enhancement quality usually obtained with their fingermarks [17, 18]. As this kind of classification is very subjective, it could lead to incorrect result assessment. A recent study proposed a donor classification based on the visual observation of fingermark residue chromatograms [15]. Using seven donors, the authors made three groups based on presence and intensities of fatty acids, squalene, cholesterol and wax esters. However, this classification remained subjective (based on visual observation) and an objective way of classifying donors based on target compounds would actually be an asset for research purposes.

Knowledge about the variability of fingermark residue is also important for the research about fingermark dating. In fact, a recent study about aging kinetics of lipid compounds highlighted the need to gain more fundamental knowledge about the initial lipid composition of fingermarks and its variability over time [14]. Furthermore, the fingermark dating research could also take advantage of an objective classification of donors based on their fingermark composition. Donors could be objectively selected in order to develop dating methodologies on a representative population.

As lipids are important targets for fingermark enhancement and dating techniques, this article mainly aimed at gathering information on these compounds through a population study of fresh fingermark residue of 25 donors. Qualitative and quantitative results were collected through GC/MS analyses and compared with the literature when possible. Based on these results, target lipid compounds were selected in order to study their variability within fingermarks of a same donor (intra-variability) and between fingermarks of different donors (inter-variability). These compounds were selected as potential target for fingermark dating

purposes, and potentially for enhancement purposes as well. Finally, a donor classification model was proposed using chemometric tools in order to objectively classify donors in groups based on these target lipids. This model was built after testing different data pre-treatments and its robustness was also tested. Such a classification could be used for fingermark dating or enhancement research.

2. Material and Methods

2.1 Sampling: donors, deposition protocol and data sets

For this study, the fingermarks of 25 different donors were collected, 13 females and 12 males, between 25 and 40 years old (except one 57 years old donor). The detailed characteristics of these donors are described in Table 1.

(TABLE 1)

All the fingermarks collected for this study were deposited in the morning on 25 mm diameter glass microfiber filters (Whatman, Bottmingen, Switzerland) according to the following deposition protocol (adapted from [15]):

1. The donors were asked to follow their tasks normally before deposition. The only condition was to avoid hand washing with soap within the last 45 minutes preceding the deposition.
2. Both thumbs were gently rubbed on the forehead and the edge of the nose, miming a natural movement.
3. The pressure and time of deposition were controlled. Each fingermark was deposited on a kitchen scale with an approximate pressure of 500 ± 20 gr during 15 seconds.
4. During each deposition session, the right and left thumbs were collected.
5. After the deposition, the donors were asked to answer a questionnaire about their habits and health in order to gather important information on donor characteristics. This questionnaire was built following the recommendations of the literature concerning the influence factors affecting the fingermark composition [6]. It was submitted and accepted by the ethic commission of Canton de Vaud in Switzerland[1].

This protocol was chosen because it optimized the fingermark composition towards donor characteristics.

Two different data sets were acquired for this work according to the deposition protocol described above:

- **Inter-variability data set**: the fingermarks of 25 donors were collected within one month, on 13 different days. Two samples per donor were collected for this data set (left and right thumbs). This number was chosen in order to collect fingermarks from the same finger (same size) at the same time to avoid interference from other influence factors.
- **Intra-variability data set**: Two donors were selected. Donor 3 (D3) deposited fingermarks on five different days within one month (for a total of 34 fingermarks) and donor 19 on six different days within one month (for a total of 38 fingermarks). A maximum of four deposition sessions were conducted per day in order to have at least two hours between each deposition. Fingermarks from the right and left thumbs were collected

[1] Commission cantonale (VD) d'éthique de la recherche sur l'être humain, Protocole 313/11, accepted in September 2011

at each deposition sessions.

The inter-variability data set was firstly used in order to conduct the qualitative and quantitative population study to gather information about lipid compounds available in fingermark residue. Based on this study, target lipid compounds were selected and their variability was evaluated and compared using both data sets. The inter-variability data set was then used to build a donor classification model using chemometrics. Finally, the intra-variability data set was used to test this classification by evaluating if fingermarks from the same donor were always classified into the same donor group.

2.2 Sample extraction and analysis

The extraction of fingermark residue occurred between 5 and 10 minutes after deposition. The microfiber filters were immersed in cap glass vials (32 x 11.6 mm, purchased from Laubscher, Geneva, Switzerland) filled with 1.5 mL dichloromethane (99.99%, purchased from Fisher Scientific, Wohlen, Switzerland) for 60 seconds and then removed. In order to concentrate the extracted residue, dichloromethane was evaporated under a stream of nitrogen until only a few microliters were left. The remaining liquid was transferred into an insert of 50 µL installed on a spring (inserts and springs purchased from Laubscher, Geneva, Switzerland) and evaporated to dryness under a stream of nitrogen. The residue was then dissolved in 20 µL of dichloromethane containing an internal standard (0.05 mg/mL of 1-decanol, purchased from Sigma-Aldrich, Buchs, SG, Switzerland). The internal standard was chosen based on its retention time and its similarity with the targeted compounds [15]. Blank samples were extracted and analysed for each analysis run.

The composition of the collected fingermarks was analysed with a gas chromatograph Clarus 500 equipped with an autosampler and coupled with a mass spectrometer Clarus 560 in electron impact (EI) mode maintained at 230°C, both instruments from PerkinElmer. The column used for the analysis was an HP5-MS, 30 m x 0.25 mm x 0.25 µm from Agilent and the carrier gas was helium with a constant flow of 1 mL/min. Sample quantity of 2 µL was injected in splitless mode using a purge time of 1.5 min. through the injector maintained at a temperature of 250°C. The temperature program was the following: 80°C during 1 min., increase from 80 to 230°C at a rate of 10°C/min, isothermic step at 230°C during 2 min., increase from 230 to 290°C at a rate of 6°C/min and then from 290 to 320°C at a rate of 3°C/min and finally, last isothermic step at 320°C during 2min. A solvent delay of 3.6min was applied and the transfer line temperature was maintained at 300°C. The mass analyser used was a quadrupole maintained at 150°C and set in scan mode between 40 and 550 m/z.

To ensure the result quality, a control solution containing one representative compound for each main lipid group (fatty acids, sterols and wax esters) and the internal standard (1-decanol, 0.05 mg/mL) was injected and analysed with the above mentioned method every week. Control charts were edited following the recommendations of the literature [19, 20]. The selected representative compounds were palmitic acid (1mg/mL), squalene (1mg/mL), cholesterol (1mg/mL), myritsyl myristate (0.1 mg/mL), and myristyl palmitoleate (0.1 mg/mL). The fatty acid and sterols were purchased from Sigma-Aldrich (Buchs, SG, Switzerland) and the wax esters from Nu-Chek Prep (Elysian, MN).

Compounds found in the chromatograms were qualitatively identified using the mass spectra information. Three different methods of identification were used:

1. Comparison with the computerized database NIST08 (Gaithersburg, MS) and/or

published data (DB).
2. Study of the mass spectra to reconstruct the molecule (MS). This method was principally used for the wax esters [15, 21, 22], as these compounds could not precisely be identified using the commercial databases. Other compounds were identified through their mass spectra when the database gave poor results and/or no standards were available.
3. Comparison of the retention time and mass spectra with standard compounds analysed using the same GC/MS method (STD).

It is important to note that the identifications made by MS have some limitations, because it is impossible to determine precisely the position of double bonds and branched carbons (i.e., isomers are not differentiable). More advanced mass spectrometry techniques should be used to obtain a precise characterization of isomers (e.g., for wax esters). This is why combinations of the three different ways of identification were often used in this work (e.g., MS + STD used for the identification of the most abundant wax esters). After the identification of each compound, target ions were selected and used for the automatic recognition of these compounds in all samples. The base peak, i.e. the most abundant peak, was selected as target ion for all compounds and if necessary, qualifier ions were used as well.

Quantitative results were obtained for palmitic acid (fatty acids), cholesterol and squalene (sterol and precursor), myristyl myristate and myristyl palmitoleate (wax esters). These compounds were selected for quantification because they are representative of the main classes of compounds found in fingermark residue. In order to obtain calibration curves, standards compounds were analysed at different concentrations and the peak areas of target ions (TI) were extracted: TI=129 for palmitic acid, TI=145 for cholesterol, TI=69 for squalene, TI=229 for myritsyl myristate and TI=236 for myristyl palmitoleate. An internal standard (1-decanol, TI = 55) was used to build the calibration curves. These calibration curves were also used to calculate the limits of detection (LoD) and quantification (LoQ) using recommendations of the literature [19]. Further details are provided in Appendix 1.

2.5 Data treatment

Pre-treatments are often used when dealing with classification purposes in order to reduce the variability of the data and allow an objective evaluation of the variability among different populations [23]. In this study, after averaging the peak areas of the two fingermark samples collected during each deposition session (right and left thumbs), four basic pre-treatments were selected based on relevant literature coming from other forensic areas, such as drug profiling or questioned ink comparison [24-31]: Normalisation to the internal standard (NormIS) and to the sum of compounds (NormSum), standardisation (STD), square root (sq. root) and logarithm in base 10 (LOG). Details about these pre-treatments are provided in Appendix 2.
Eight combinations of these five pre-treatments were used, applying normalisation in each combination as this pre-treatment allows minimising errors of sample preparation and analysis: (1) NormIS, (2) NormSum, (3) NormIS+STD, (4) NormIS+sq.root, (5) NormIS+LOG, (6) NormSum+STD, (7) NormSum+sq.root and (8) NormSum+LOG (absolute values). In order to study the variability of target compounds, the first two pre-treatments were applied, allowing an objective evaluation and comparison. Then, in order to obtain a robust donor classification, the eight combinations of pre-treatments were tested combined with measurements of similarity (Pearson correlation) and dissimilarity (Euclidean distance) [23, 25, 26]. In this study, the best results were obtained using Euclidean distance

and as such, only this type of measurements is reported in the results.

Receiver Operating Characteristics (ROC) curves were used to evaluate the efficiency of the combinations of the above-described pre-treatments with Euclidean distance, in order to discriminate between two distributions: fingermarks of the same donor and fingermarks from different donors. ROC curves are built by plotting the *true positive rate* (sensitivity) against the *false positive rate* (1-specificity). The value of the *area under the ROC curve (AUC)* allows identifying the best metrics for a given purpose, i.e. generally allowing the best discrimination between two populations. AUC values range from 0.5 (distributions completely overlapped) to 1 (distribution completely separated) [32].

Finally, in order to develop a classification model for fingermark donors based on target lipid compounds, a hierarchical cluster analysis (HCA) was conducted. HCA is an unsupervised classification method allowing object grouping based on distance measurements. Different solutions can be used in order to group object using HCA: divisive or agglomerative clustering linkage. By the former, clustering starts with a single cluster, containing all samples, which is successively divided into smaller clusters. By the latter, clustering starts with single objects and joins them successively together according to the distance between them in order to build larger clusters. The complete linkage clustering is the more common algorithm used for agglomerative clustering [33]. The result of this type of clustering is visible using dendrograms [23, 33]. In this study, different types of agglomerative linkages clustering (single, average, median and complete linkage) and different distance measurements (Pearson and Euclidean) were tested. However, the best results were obtained using complete linkage clustering combined with Euclidean distance and as such, only this type of clustering is reported in the results.

To conduct the above-mentioned steps, different softwares were used. Pre-treatments and distance measurements were computed using Microsoft Excel® 2011 for Mac (Microsoft Corporation), the ROC curve were built using SPSS® Statistics 20 for Mac (IBM Corporation) and HCA was conducted with The Unscrambler® X 10.3 (Camo Process AS).

3. Results and discussions

3.1 Qualitative population study

104 compounds were detected in this study (Table 2):

- 91 lipids: 13 fatty acids, 76 wax esters, cholesterol and squalene (sterol and precursor)
- 8 lipid derivatives: 6 oxidation products of squalene, 1 cholesterol ester and 1 cholesterol intermediate
- 4 benzoic acid esters
- 1 vitamin: gamma-tocopherol, the natural molecule of vitamin E

A typical fingermark residue chromatogram is shown in Figure 1, together with the temperature gradient used for the GC method.

(FIGURE 1)
(TABLE 2)

3.1.1 Fatty acids

The fatty acids identified in this work include saturated and unsaturated molecules containing from 8 to 18 carbon atoms. All these compounds were mentioned in a recent review about fingermark composition [1]. Other fatty acids containing from 17 to 24 carbon atoms were previously detected, but were not observed in the samples analysed in the present study. This absence can be explained through differences in the sample preparation and the analytical technique. In fact, three of the publications mentioning these large fatty acids added derivatizing agents during the sample preparation [5, 10, 11], and another publication applied MALDI/MS instead of GC/MS [34].
All identified fatty acids were present in a large majority of the analysed fingermarks (>85%) and eight were actually present in all fingermarks and donors: nonanoic acid (9:0), decanoic acid (10:0), myristoleic acid (14:1), myristic acid (14:0), palmitoleic acid (16:1), palmitic acid (16:0), oleic acid (18:1) and stearic acid (18:0). The identified fatty acids are fingermark endogenous compounds as they originate from the epidermis (hydrolipidic film covering the horny layer) and the sebaceous glands [1]. However, all these fatty acids can be extrinsic contaminants as well, as all of them can be found in cosmetics based on the European Commission database about cosmetic substances and ingredients (Cosing[2]) and in relevant literature [35-37]. Fatty acids can thus be particularly interesting for enhancement purposes in order to obtain a visible reaction with target compounds whose origin and exact amounts are not the main focus. However, fatty acids seem to be rather poor targets for dating purposes. Indeed, information about the presence of cosmetics in a question fingermark will be very difficult to obtain and the study of their aging kinetics may thus be biased.

3.1.2 Wax esters

Wax esters originate from the sebaceous glands and result from the esterification between a fatty acid and a fatty alcohol. In this study, numerous wax esters were identified including saturated and unsaturated aliphatic fatty acids containing between 11 and 16 carbon atoms and saturated fatty alcohols with 3 to 21 carbon atoms (branched or not). Only one recent study focused on wax ester detection and reported 29 wax esters in fingermark residue [15], all of which were identified in this work, along with 23 additional ones. These 23 wax esters have never been mentioned previously in the literature concerning fingermark composition. Among them, four isopropyl molecules were identified through the database and the analysis of their mass spectra: isopropyl dodecanoate, isopropyl tetradecanoate (myristate), isopropyl-12-methyl-tetradecanoate (myristate) isopropyl-hexadecanoate (palmitate). 19 were identified using their mass spectra alone[3]. The identification of these additional wax esters can be explained by the optimization of the GC/MS method and by the increased number of donors. It should be noted that numerous wax ester isomers have also been found in this study (particularly from the esterification of myristic or palmitic acid with myristyl or palmityl alcohol[4]). However, it is not possible to differentiate isomers using GC/MS [22] and it was thus difficult to know whether each isomer had already been mentioned in the literature or not. This is the reason why all isomers were considered as a group and if one of them had already been mentioned in the literature, none of them were counted as additionally identified.

10 wax esters were identified in all analysed fingermarks: isopropyl dodecanoate, isopropyl tetradecanoate (myristate), myristyl myristoleate, myristyl palmitoleate and myristyl palmitate, palmityl palmitoleate and palmityl palmitate, WE[17:0, 16:1] (Rt=33.088), stearyl

[2] http://ec.europa.eu/consumers/cosmetics/cosing/, last access on the 13th december 2013
[3] WE(10:0, 11:0), WE(14:0, 12:0), WE(14:0, 13:0), WE(14:0, 15:1), WE(14:0, 17:0), WE(15:0, 16:0), WE(17:0, 15:1), WE(18:0, 14:1), WE(18:0, 15:1), WE(17:0, 16:0), WE(18:0, 15:0), WE(19:0, 15:1), WE(20:0, 14:0), WE(20:0, 14:1), WE(19:0, 16:1), WE(21:0, 15:0), WE(22;0, 14:0), WE(20:0, 16:0) and WE(21:0, 16:1)
[4] WE(14:0, 14:0), WE(14:0, 15:1), WE(14:0, 15:0), WE(14:0, 16:1), WE(16:0, 16:1), WE(16:0, 16:0), WE(18:0, 16:1), WE(20:0, 15:0), WE(20:0, 16:1) and WE(21:0, 16:1)

palmitoleate and WE[20:0, 16:1] (Rt=37.663). The fatty acid parts of these wax esters correspond to the fatty acids identified in all donor fingermarks. Other large wax esters were found in a very limited number of fingermarks[5]. These large wax esters were present in fingermark residue coming from donors showing high amounts of wax esters in general. All the wax esters found in this study are fingermark endogenous compounds, but some of them are also used in the cosmetic industry. In fact, isopropyl myristate and isopropyl palmitate, as well as wax esters made from the esterification of myristic or palmitic acid with myristyl or palmityl alcohol can be encountered in some emollients[6]. However, the most common wax esters found in cosmetics are larger ones, not identified in fingermark residue: triacontanyl palmitate ((30:0, 16:0), main constituent of beeswax) and wax esters containing from 40 to 42 carbon atoms (main constituents of jojoba oil). All wax esters are thus good targets for enhancement purposes, for the same reason as the fatty acids. Furthermore, wax esters being rarely used in the industry are also interesting for dating purposes, because they are reactive compounds and their aging kinetics may yield useful information for this particular field.

3.1.3 Squalene and cholesterol

Squalene is the precursor of cholesterol and both compounds were found in all fingermarks and donors. Squalene is a very reactive non-volatile triterpene present in the sebum and containing numerous unsaturated moieties. It can thus be easily oxidized through UVA, UVB, environmental oxidants (e.g., ozone) and microbes [38, 39]. This is the reason why oxidation products of squalene have already been identified in fresh fingermark residue [5, 40]. Based on Montfort's work, the most common oxidation products of squalene in fresh fingermarks are squalene monohydroperoxides and squalene peroxides. However, smaller oxidation products as aldehydes and ketones can also be produced by ozonolysis of squalene [39]. In fact, the oxidation of squalene through reaction at bond 1, 1', 2, 2', 3 and 3' can produce acetone, 6-methyl-5-hepten-2-one (6-MHO), 4-OPA, geranyl acetone (GA), and long-chain aldehydes (ozonolysis mechanism postulated by Petrick [39]). In this study, the four following oxidation products of squalene were identified in all fingermarks based on their mass spectra and comparison with the oxidation products described by Petrick [39]:

- 5,9-Undecadien-2-one,6,10-dimethyl-, (E)- (Geranyl acetone, GA)
- 5,9,13-trimethyl-tetradeca-4,8,12-triene-al (TTT)
- 4,8,13,17,21-tetra-methyl-octadeca-4,8,12,16,20-pentaene-al (TOP)
- 4,9,13,17-tetramethyl-octadeca-4,8,12,16-tetraeneal (TOT)

Two additional oxidation products of squalene were found in fingermark residue (Rt=27.47 and 27.53 min). As no identification based on the literature could be made, these compounds were classified as "possible" squalene oxidation products because their spectra contained specific ions of squalene (69 and 81).

The cholesterol present in fingermark residue may originate from two distinct sources. The first one is the sebum, which is reached by cellular cholesterol located in the plasma through blood circulation. The second one is the epidermis, more particularly the hydrolipidic film covering the horny layer [1]. Cholesterol undergoes oxidation because of the presence of a double bond in position 5,6 of the B ring. Cholesterol oxidation follows similar pathways as monounsaturated fatty acids and the following hydroperoxycholesterols (HPC), hydroxycholesterols (HC), ketocholesterol (KC) and epoxycholesterol (EC) have been identified as cholesterol oxidation products: 5α-HPC, 6α-HPC, 6β-HPC, 7α-HPC, 7β-HPC, 7α-HC, 7β-HC, 7-KC, 5α-6α- EC, 5β-6β-EC [41, 42]. Despite its oxidation capacity, no cholesterol oxidation products were detected in this study. However, a cholesterol ester

[5] WE(18:0, 15:1), WE(18:0, 16:1), WE (19:0, 15:1), WE(20:0, 16:1). WE(20:0, 15:0), WE(21:0, 16:0) and WE(21:0, 16:1)
[6] http://ec.europa.eu/consumers/cosmetics/cosing/, last access on the 13th of december 2013

(cholest-5-en-3-ol-propanoate or cholesteryl propionate, Rt=26.93 min) was identified in the analysed fingermarks. This molecule can be the result of side reactions between cholesterol and other compounds (e.g. fatty acids) [43]. It can also be present in fingermark residue because it is the storage form for cholesterol when there is an excess of intracellular cholesterol in membranes [44]. Lanosterol (Rt=31.634) was also found in the fingermark residue analysed in this study. The presence of this molecule may be explained because it is an intermediate in cholesterol biosynthesis [45]. Identification of these molecules was based on their mass spectra, comparison with the database and information found in the literature.
Squalene and cholesterol are rarely found in cosmetics, but can be present in some emollients and hair conditioning products[7]. Concerning their oxidation products or other derivatives, none are mentioned in cosmetic lists. Squalene, cholesterol and their oxidation/derivation products are thus interesting targets for enhancement purposes and can be promising as well for dating purposes.

3.1.4 Other compounds

Oxidation happens at the surface of the skin, as mentioned above. However, in order to prevent too much oxidation, the skin is equipped with enzymatic and non-enzymatic antioxidant systems. Vitamin E was identified as predominant antioxidant in the uppermost human skin layers, the stratum corneum and skin surface lipids [42]. The most biologically active form of Vitamin E, the alpha-tocopherol, is thus used in numerous cosmetics[7]. In this study, the gamma-tocopherol form of vitamin E was found in the fingermarks of donors 2 and 17 (Rt=28.60). These donors reported the use of, respectively, foundation crème and face crème. However, gamma-tocopherol was not found in the composition of these cosmetics. Its origin (contaminant or endogenous compounds) is thus unsure.

Finally, major alkanes containing from 25 to 32 carbon atoms were also found in some fingermarks analysed for this study (Figure 2). These compounds appeared and disappeared unregularly during the analyses and the presence of some kind of contaminations (from plastics, rubbers or mechanical waxes) could not be excluded. Therefore, they were not considered as fingermark endogenous compounds and not mentioned in Table 2. However, even if the origin of these compounds remains unclear, it is interesting to note that these alkanes correspond to the ones identified by Bortz in his study about human skin surface lipids [46]. Therefore, further studies should be conducted in order to precisely identify the source of such alkanes in trace analysis.

(FIGURE 2)

3.2 Quantitative population study

Palmitic acid (PALM), squalene (SQUAL), cholesterol (CHOL), myristyl myristate (MM) and myristyl palmitoleate (MPO) were chosen for quantification because they represent the main categories of lipids identified in this work and were detected in all fingermarks. To the best of our knowledge, a few studies already quantified PALM [5, 8, 11, 47], SQUAL [5, 8, 11, 14, 47] and CHOL [5, 48] in fingermark residue, but no quantitative information is available about MM and MPO yet. Table 3 summarizes the quantitative results obtained in this study, expressed in nanogram (ng) per fingermark (FM), as well as the standard deviation of each concentration S_{x0} (i.e., the error calculated from the calibration curves).

(TABLE 3)

[7] http://ec.europa.eu/consumers/cosmetics/cosing/, last access on the 13th of december 2013

Five values of palmitic acid concentrations were under the limit of quantification (LoQ) and were thus not included in Table 3 (fingermarks of donors 11, 15, 20 and 21). The lowest concentrations above the LoQ belonged to one fingermark of donors 15 and one of donor 22 (respectively 169.19 and 177.18 ng/FM). The maximum values were found in the fingermarks of donor 6 (13'761.86 and 11'334.81 ng/FM) and were much higher than the concentrations of found in the fingermarks of the other donors. Fingermarks of donor 5 yielded the second maximal concentrations (3'107 and 2'646.10 ng/FM). The palmitic acid concentrations obtained in this study are similar to previously published data, if donor 6 is not considered. Indeed, the literature mentioned concentration from 75 to 1'637 ng/FM [5, 8, 11]. No particular parameters could explained the high values obtained for donor 6, except the fact that this donor wore hair gel. However, it was not possible to control the content of this cosmetic, as the donor did not remember the brand used. This observation highlighted the large quantitative inter-variability among fingermark residue.

The concentrations of squalene were above the LoQ in all fingermarks, except for one value between the LoD and LoQ (belonging to donor 20, not shown in Table 3). The minimal concentrations being above the LoQ were found in one fingermark of donor 21 and one fingermark of donor 20 (respectively 78.76 and 89.78 ng/FM). The maximum concentrations were again found in fingermarks of donor 6 (5'662.56 and 5'280.42 ng/FM), followed by one fingermark of donors 18 and 7 respectively (4'772.05 and 4'633.38 ng/FM). The concentrations found in this study were comparable to previously published data showing concentration between 28 to 5'311 ng/FM [5, 8, 11, 14].

The concentration of cholesterol was found to be above the LoQ in all fingermarks. The minimal values were obtained in fingermarks of donor 21 (76.71 and 92.22 ng/FM) while donor 17 showed this time the maximal concentrations (978.17 and 910.55 ng/FM), followed by one fingermark of donors 6 and 9 respectively (703.87 and 701.05 ng/FM). The only cholesterol concentration mentioned in the literature is 1,032 ng/FM [5] and is thus comparable with the values obtained in this study. Donor 17 showed high concentration values in comparison with the other donors. While this donor reported the use of face crème, no cholesterol was found in its composition. A possible explanation may be that donor 17 suffers from hypercholesterolemia (see Table 1). In fact, his last medical control identified relatively high cholesterol values but this diagnosis is not confirmed yet and donor 17 is not treated against this disease. On the contrary, donor 21 showed the minimal concentrations of cholesterol while suffering from hypercholesterolemia (confirmed diagnosis). However, this donor is treated against this disease. In case of hypercholesterolemia, high levels of cholesterol are found in the blood and are thus present in the blood plasma, which reaches the sebum through blood circulation. As the sebum is one source of cholesterol in fingermark residue, a high level of cholesterol in the plasma can result in a higher level of cholesterol in fingermark residue. This hypothesis should be further tested in order to determine what kind of relation exists between the concentration of cholesterol in blood and fingermark residue cholesterol.

The concentrations of myristyl myristate of six fingermarks were below the LoQ and are thus not considered in Table 3 (donors 15, 20 and 22). The minimal values above the LoQ were obtained with one fingermark of donor 11 and 16 respectively (4.33 and 4.54 ng/FM). The maximal values were obtained with one fingermark of donor 6 and 9 respectively (70.62 ng/FM and 68.39 ng/FM).

The myristyl palmitoleate concentrations of five fingermarks were under the LoQ and are thus not considered in Table 3 (donors 15, 20 and 21). The minimal concentrations being above the LoQ belonged to one fingermark of donor 21 and 8 respectively (6.56 ng/FM and 13.46 ng/FM). The maximum concentrations were found in the fingermarks of donors 6 (1023.97 and 713.70 ng/FM) and 17 (241.00 and 218.15 ng/FM). As for the palmitic acid concentrations, no parameters could explain the high values obtained for donors 6, except the possible presence of hair gel whose exact composition could not be controlled.
No reports of myristyl myristate and myristyl myristoleate concentrations in fingermark residue were found in the literature for comparison.

It is interesting to highlight that fingermark from the same donors gave the lowest concentrations for all compounds: donors 15, 20, 21 and 22. The same was also observed for the largest concentrations: fingermarks of donor 6 contained the highest concentrations of each compound except cholesterol and fingermarks of donors 5, 7, 9, 17 and 18 were generally the most concentrated samples. These observations tend to show that the amounts of different lipids are correlated among one fingermark. It is also interesting to note that some diseases influenced lipid content. In fact, hypercholesterolemia seemed to increase cholesterol amounts in fingermarks when no treatment was taken. Furthermore, donor 7 reported suffering from acne and his fingermarks contained high amounts of all lipids, particularly fatty acids, what corresponds to a previous study [15].

3.3 Evaluation of the variability of target lipid compounds

In order to assess and compare the intra-variability (variability among fingermarks of a same donor) and inter-variability (variability between fingermarks of different donors) of fingermark residue, target lipid compounds were selected. These compounds could be good targets for the research about fingermark dating or enhancement, as their selection was based on the following criteria: (1) presence in all analysed fingermarks, (2) no usual constituents of common fingermark contaminants (e.g., cosmetics), (3) reasonable abundance in all the tested samples (signal to noise ratio: S/N > 10) and (4) good resolution. The following 10 compounds were thus chosen (highlighted in Table 2):

- Isopropyl dodecanoate (IPD)
- Squalene (SQUAL)
- Cholesterol (CHOL)
- Myristyl palmitoleate (MPO)
- Myristyl palmitate (MP)
- Palmityl palmitoleate (PPO)
- Palmityl palmitate (PP)
- Wax ester 1 (WE[17:0, 16:1], Rt=33.088) (WE 1)
- Stearyl palmitoleate (SPO)
- Wax ester 2 (WE[20:0, 16:1], Rt=37.663) (WE 2)

The peak area of each target compounds was extracted from all collected fingermarks. The average of these peak areas was then calculated between the two fingermarks collected at each deposition session and normalisation was applied. In order to evaluate the variability objectively, the relative standard deviations (RSD) were finally calculated (Figure 3):

- For the intra-variability of fingermark residue from donors 3 (17 averages obtained from 34 fingermarks) and donor 19 (19 averages obtained from 38 fingermarks)
- For the inter-variability of fingermark residue from 25 different donors (25 averages obtained from 50 fingermarks).

The RSD values calculated from normalisation to the internal standard are reported in Figure 3A. The RSD values obtained for intra-variability were much lower than for inter-variability concerning the larger wax esters (i.e., from MPO to WE2). In fact, the RSD ranged from 77 to 126% for intra-variability while it ranged from 130 to 203% for inter-variability. Concerning the other target compounds, RSD values were more similar between intra and inter-variability as they ranged from 44 to 94 % and from 63 to 90 % respectively. It is interesting to note that donor 3 generally showed higher RSD values than donor 19, even if mainly lower than the inter-variability. While the intra-variability was comparable to past studies [14, 15], the inter-variability showed higher RSD values. This observation may be explained by the difference in the number of donors used (25 in this study against six or seven in the previous studies) and actually confirmed the large variability of lipid amounts between different donors.

When normalisation to the sum of the target compounds (excluding squalene) was applied (Figure 3B), the RSD values of intra and inter-variability were reduced for all compounds in comparison with the normalisation to the internal standard, except for IPD. An explanation for this observation could be that IPD amount was influenced by different factors than the other compounds, what can explain this increase in variability when the normalisation to the sum was used. If IPD is chosen for research purposes, the cause of this variability should be further investigated. Furthermore, concerning PP, the RSD value of intra-variability for donor 3 was higher than for inter-variability. This observation can be explained by the fact that the amount of PP in donor 3 was rather low in comparison with the other compounds and the RSD was thus greatly influenced by the normalisation to the sum. It is also interesting to note that RSD values were similar for all compounds. The difference between large wax esters and the other compounds was not significant anymore using the normalisation to the sum. In fact, the RSD values of all compounds ranged from 25 to 95 % for the intra-variability and from 48 to 143 % for the inter-variability (maximum value for MP).

This comparison of the intra and inter-variability showed that, in general, the RSD was significantly lower for the intra-variability, what corresponds to past studies [14, 15]. It was also shown that the pre-treatment used had an influence on the results. In fact, the normalisation to the sum was able to reduce the intra and inter-variability of nearly all compounds in comparison with the normalisation to the internal standard.

(FIGURE 3)

3.4 Proposition of an objective donor classification model

3.4.1 Choice of pre-treatments

In order to develop an objective donor classification model, the present study used 10 target compounds found in the fingermark residue of 25 donors. Before building the classification model itself, eight different pre-treatments were tested on the data sets, combining normalisation to the internal standard (NormIS) and to the sum without squalene (NormSum) with square root (sq.root), logarithm (LOG, absolute values) or standardisation (STD). The choice of the best pre-treatment to build a classification model was based on the following two objectives:

- **Objective 1:** Reach an optimal separation of the intra and inter-variability distributions, in order to be able to differentiate between fingermarks of a same donor and fingermarks from different donors. The donor classification will be robust if fingermarks of a same donor are always classified into the same group. In order to find the metric fulfilling this

objective, the eight pre-treatments were applied on the data sets and followed by Euclidean distance calculation.

The results were studied using ROC curves (see Figure 4). According to Fawcett [32], the metrics yielding AUC values between 0.5 and 0.7 offer a worthless or poor separation. Thus, only two combinations did actually yield a fair separation over 0.7 when a confidence interval of 95% was taken into consideration: NormIS (0.711-0.788) and NormSum+LOG (0.718-0.794) (bold and highlighted in Figure 4). It is important to note here that a fair separation is probably the best possible result under the tested conditions. As the aim of this work was to propose a classification model usable for fingermark dating or enhancement research, the 10 target compounds were selected particularly with regard to their presence in all fingermarks samples. If compounds only available in fingermarks of some donors had been chosen, the separation would have been better. However, the obtained classification would have been useless for fingermark dating or enhancement research because compounds only available in the fingermarks of some donors would never be targeted for such purposes.

- **Objective 2:** Scale the target compounds on a comparable magnitude. Indeed, relative proportions critically influences distance calculation and separation using chemometric tools [49]. If some compounds have a much larger magnitude, they also have a larger influence on the separation. In order to fulfil this aim, the pre-treatments pre-selected through the first objective were used to build boxplots of each compound and study their relative proportions.

The results showed that squalene had a much larger magnitude than the other compounds using NormIS (Figure 5A). Apart from squalene the relative proportions of other compounds were comparable. However, using this pre-treatment, extreme values were very much spread out for each compound. On the contrary, NormSum+LOG (Figure 5B) yielded similar relative proportions between the 10 target compounds. Furthermore, the extreme values were not spread out, resulting in an optimal result according to the two objectives. NormSum+LOG was thus selected in order to build a donor classification model.

(FIGURE 4)
(FIGURE 5)

3.4.2 Classification using hierarchical clustering analysis (HCA)

In order to classify donors in groups based on the 10 selected target compounds, HCA was conducted after applying the selected pre-treatment (NormSum+LOG) and using complete linkage clustering with Euclidean distance. The obtained dendrogram allowed the detection of two clearly separated main groups (Figure 6):

(A) Donors 14, 15, 20, 21, 22 and 25.
This main group can be further separated into two sub-groups: (a1) donors 15, 20 and 25 and (a2) donors 14, 21 and 22, corresponding respectively to “poor” and “medium-poor” lipid donors.

(B) Donors 1-13, 16-19, 23 and 24.
This main group can also be differentiated into two additional sub-groups: (b1) donors 2, 5, 6, 7, 9, 17 and 23 and (b2) donors 1, 3, 4, 8, 10, 11, 12, 13, 16, 18, 19 and 24, corresponding respectively to “rich” and “medium-rich” lipid donors.

Chromatograms were selected to illustrate each group (Figure 7). This figure also showed that while the classification model is only based on 10 target compounds, the whole lipid content was visibly correlated to the classification. Indeed, fingermarks of "rich" donors (b1) contained larger amount of all lipids and fingermarks of "poor" donors (a1) showed lower amount of all lipids. Furthermore, the two main groups (A) and (B) actually corresponded to the quantification results presented above. In fact, donors 15, 20 and 21 yielded fingermarks containing the minimal amounts of the four quantified compounds and were classified together as "poor" donors (group A), while donors 5, 6, 7, 9, 17 and 18 showed large amounts of the quantified compounds and were classified together as "rich" donors (group B). These observations supported the fact that the proposed classification illustrated real differences between fingermark residue from different donors based on general lipid content.

(FIGURE 6)
(FIGURE 7)

In order to study the robustness of the proposed model, the intra-variability data set gathering respectively 17 and 19 fingermark replicates of donor 3 and 19 was introduced into the classification model to test if fingermarks of the same donor were always classified into the same group. Previously, fingermarks of donor 3 and 19 were classified into main group B and sub-group b2 (Figure 6).

A new dendrogram was thus built (Figure 8) and showed that a majority of the fingermark replicates of donor 3 and 19 were classified into the correct main group B (highlighted in light green in Figure 8 and Table 4). However, five exceptions were classified into main group A (highlighted in dark red in Figure 8 and Table 4). These false classifications were probably due to the intra-variability of each donor, as no external factors could explain the differences showed by these replicates. Both donors gave similar results, as Donor 3 reached a correct classification rate in more than 80% of the cases (14 over 17 replicates) and donor 19 in nearly 90% of the cases (17 over 19 replicates) Overall, a correct classification into the two main groups was reached in about 86% of the cases (31 over 36 replicates).

Main groups A and B could be further separated into four sub-groups corresponding to a1, a2, b1 and b2 (Figure 8 and Table 4). These sub-groups were very similar to those in Figure 6. However, fingermarks of donors 12 and 18 were classified into b1 in Figure 8 instead of b2 in Figure 6. This difference showed that introducing the replicates of donor 3 and 19 in the model actually influenced the separation. Furthermore, when studying the classification of fingermark replicates among the four sub-groups, 6 additional fingermarks were wrongly classified into b1 instead of b2 (Figure 8). Donor 3 reached a correct classification rate in above 75% of the cases (13 over 17 replicates) and Donor 19 obtained more than 60% of correct classification (12 over 19 replicates). Donor 3 showed thus a smaller intra-variability than Donor 19 concerning sub-groups. Overall, a correct classification into the four sub-groups was reached in about 69% of the cases (26 over 36 replicates).

As demonstrated, the proposed classification model represents a particularly useful way to objectively classify and select different types of donors for research purposes. The pool of 25 donors could be reproducibly classified into "poor" and "rich" lipid donors with a relatively low false classification rate (about 14%). Furthermore, a more precise classification into four sub-groups called "poor", "medium-poor", "medium-rich" and "rich" lipid donors was also possible with acceptable false classification rate (about 31%).

(FIGURE 8)

(TABLE 4)

4. Conclusion and perspectives

Lipids available in fingermark residue play an important role in forensic science research. They represent interesting target compounds for enhancement techniques and may also be useful in the development of a fingermark dating methodology. This work proposed a comprehensive population study of the lipids available in fingermark residue of 25 donors using GC/MS and allowed the identification of 104 compounds. Among them, 43 were reported for the first time as endogenous fingermark compounds. These were mainly wax esters (32), squalene oxidation products (4), benzoic acid esters (4), cholesterol esters (2) as well as gamma-tocopherol, the natural form of vitamin E.

Quantitative data about fingermark residue were also collected during this study. While the quantities detected for palmitic acid, squalene and cholesterol corresponded to those reported in the literature, quantitative information about wax esters (myristyl myristate and myristyl palmitoleate) was collected for the first time. Palmitic acid and squalene showed the larger amounts in all fingermarks, followed by cholesterol, myristyl palmitoleate and myristyl myristate. Furthermore, the amount of these compounds proved to be correlated within fingermark residue. Finally, it was observed that hypercholesterolemia and acne affected the lipid content.

Ten target lipids were then selected based on their presence in all fingermark samples. These were moreover rarely encountered in contaminants such as cosmetics and could thus be interesting targets for enhancement and dating purposes. The variability of these compounds was studied among fingermarks from the same donor (intra-variability) and between fingermarks of different donors (inter-variability). It was shown that their relative standard deviation was significantly lower for the intra-variability than for the inter-variability. However, it was observed that the use of data pre-treatments could significantly reduce both kind of variability. This observation could be particularly useful for the development of a fingermark dating methodology in order to reduce the variability of fingermark residue, which was previously identified as the main drawback for the development of such a methodology [14, 15].

Finally, several statistical pre-treatments were tested in order to propose an objective donor classification model based on the previously selected target lipid compounds. Normalisation to the sum (apart from squalene) followed by the logarithm gave the best results. Hierarchical cluster analysis (HCA) was then applied on the pre-treated data and it was possible to classify the fingermarks of the 25 donors in two main groups corresponding to “poor” and “rich” lipid donors. These two main groups could be sub-divided more finely in four sub-groups (i.e., “poor”, “medium-poor”, “medium-rich” and “rich”). In order to test the robustness of the proposed classification, numerous fingermark replicates from two donors (deposited within one month) were added to the classification and more than 86% of these fingermarks were correctly classified into the two main group. Furthermore, a more precise classification into the four sub-groups was also possible with a slightly higher false classification rate (up to 31%). Such a model could be particularly useful for research and development purposes, because it allows an objective classification and selection of representative donors based on target compounds of interest. The robustness of such a model should be further tested and other compounds (e.g., additional lipids or amino acids) could be used, according to the research purposes.

5. References

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Figure 1: Example of chromatogram of fingermark residue (donor 19)**.** The main lipid classes are indicated and the line represents the temperature gradient of the GC analysis.

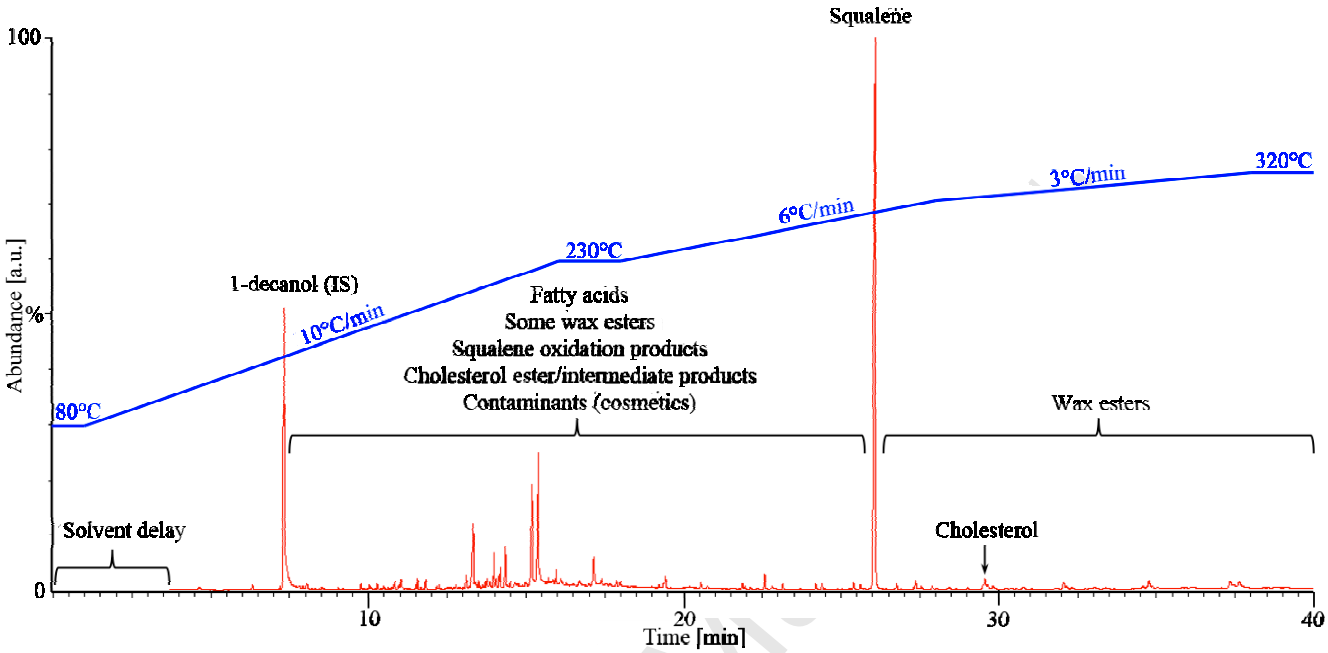


Figure 2: Example of a chromatogram containing alkanes (C25-C32).

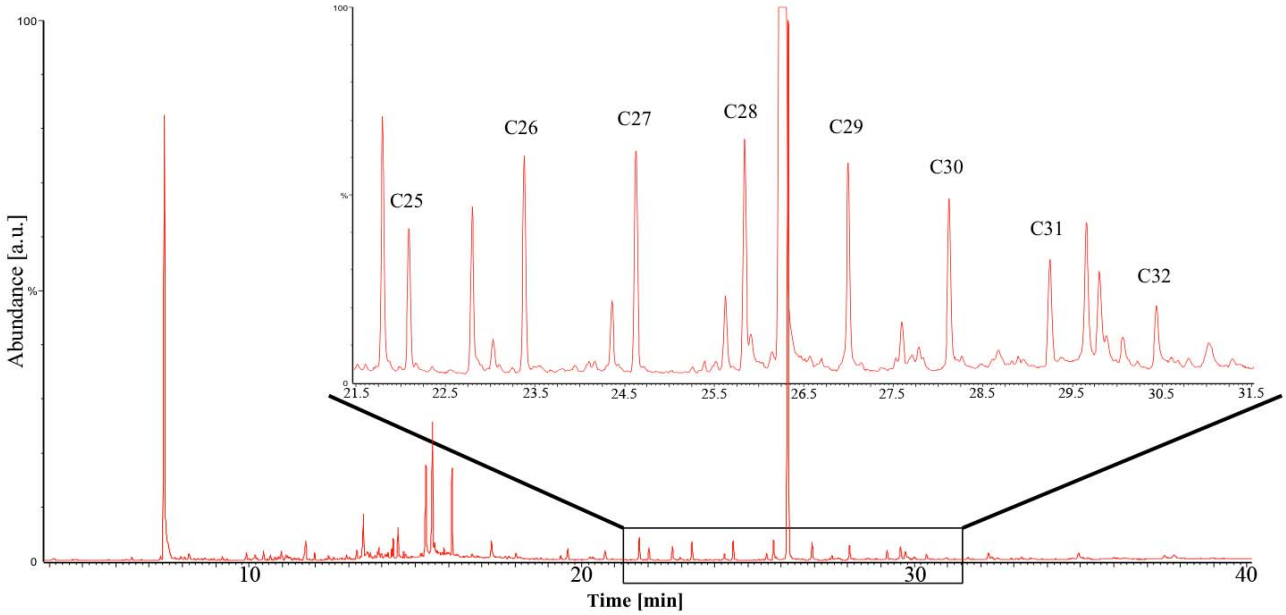


Figure 3: Comparison of the relative standard deviations (% RSD) of 10 target compounds for the variability within fingermarks of Donor 3 (*D3*) and Donor 19 (*D19*) (*intra-variability or intra*) and between fingermarks of 25 different donors (inter-variability or inter): *(A) normalisation to the internal standard (Norm.IS), (B) normalisation to the sum without squalene (Norm.Sum).*

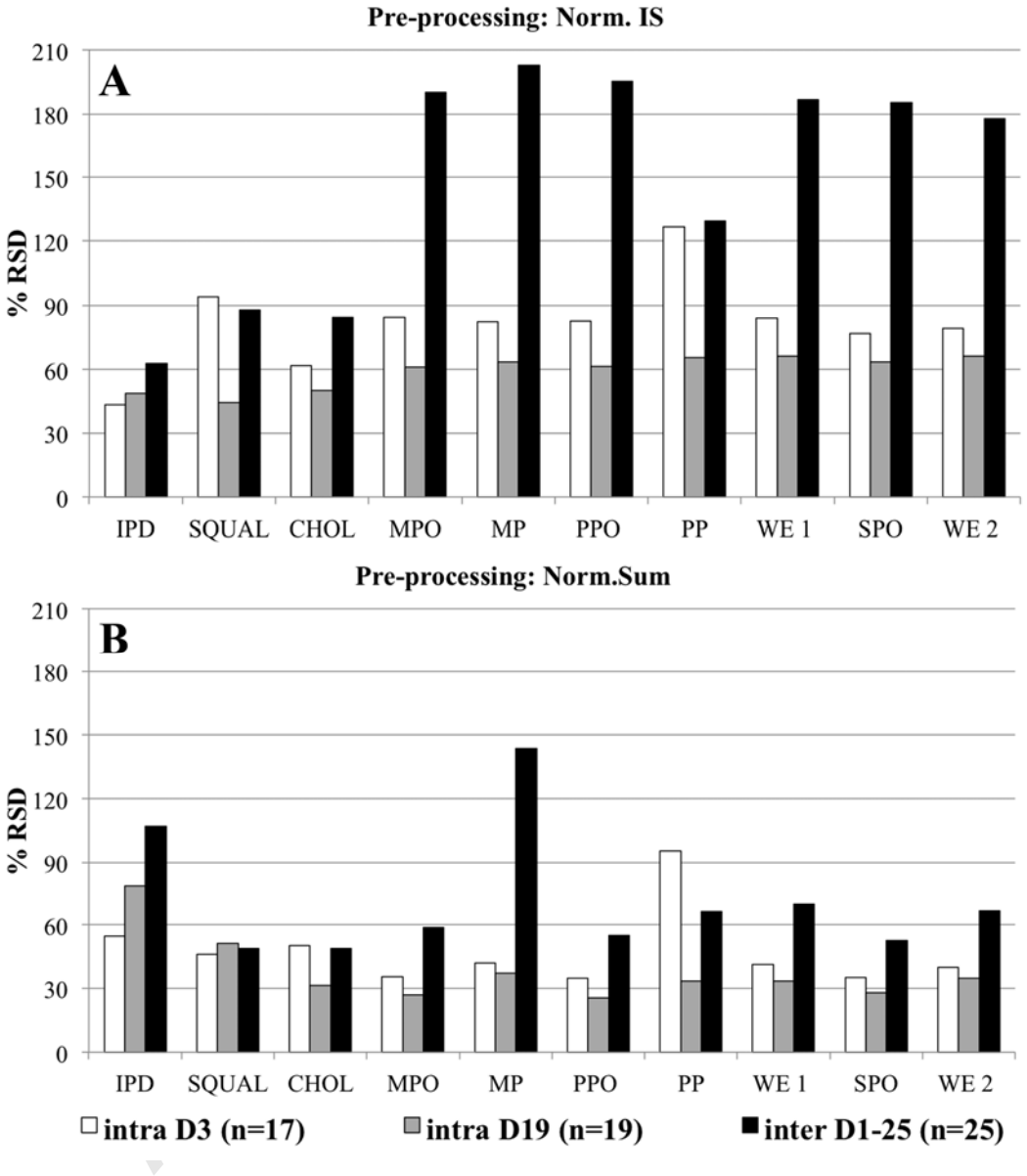
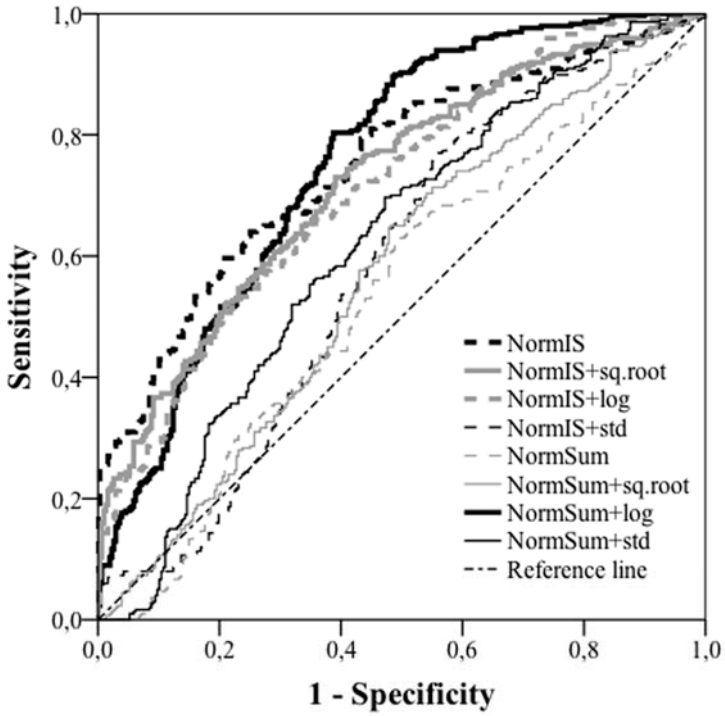


Figure 4: ROC curves and separation parameters between intra and inter-variability distributions using different pre-treatments coupled with Euclidean distance. The **bold and highlighted** metrics offered a significant separation and acceptable errors using a confidence interval of 95%.



Pre-treatments	Area	Standard error	Asymptotic significance	Asymptotic confidence interval 95%	
				Lower bound	Upper bound
NormIS	**0.750**	**0.020**	**0.000**	**0.711**	**0.788**
NormIS + sq. root	0.724	0.020	0.000	0.684	0.764
NormIS + log	0.713	0.020	0.000	0.673	0.754
NormIS + std	0.582	0.023	0.000	0.536	0.627
NormSum	0.538	0.024	0.105	0.492	0.584
NormSum + sq. root	0.570	0.023	0.003	0.524	0.615
NormSum + log	**0.756**	**0.019**	**0.000**	**0.718**	**0.794**
NormSum + std	0.620	0.023	0.000	0.575	0.664

Figure 5: Relative proportions of the 10 target compounds among fingermarks of donor 3 (intra D3), fingermarks of donor 19 (intra D19) and fingermarks from 25 different donors (inter) using two different pre-treatments: (A) Normalisation to the internal standard and (B) Normalisation to the sum without squalene followed by the logarithm (absolute values).

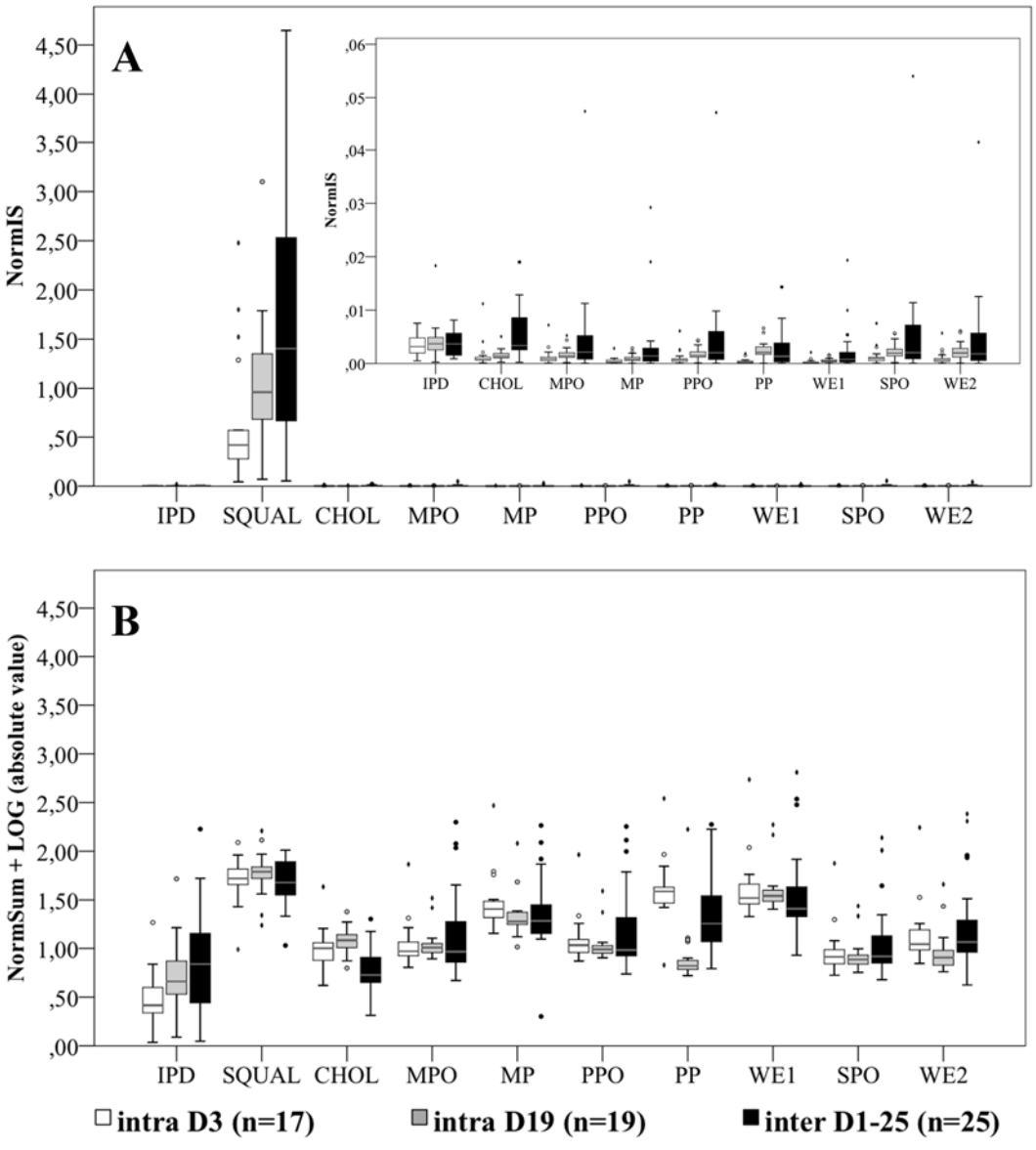


Figure 6: Classification of the 25 donors using hierarchical cluster analysis with Euclidean distance and complete linkage clustering. The normalisation to sum followed by the logarithm (NormSum+LOG) was applied as pre-treatment. Two major clusters were clearly visible (A and B) and were further separated into four sub-groups (a1 and a2) and (b1 and b2).

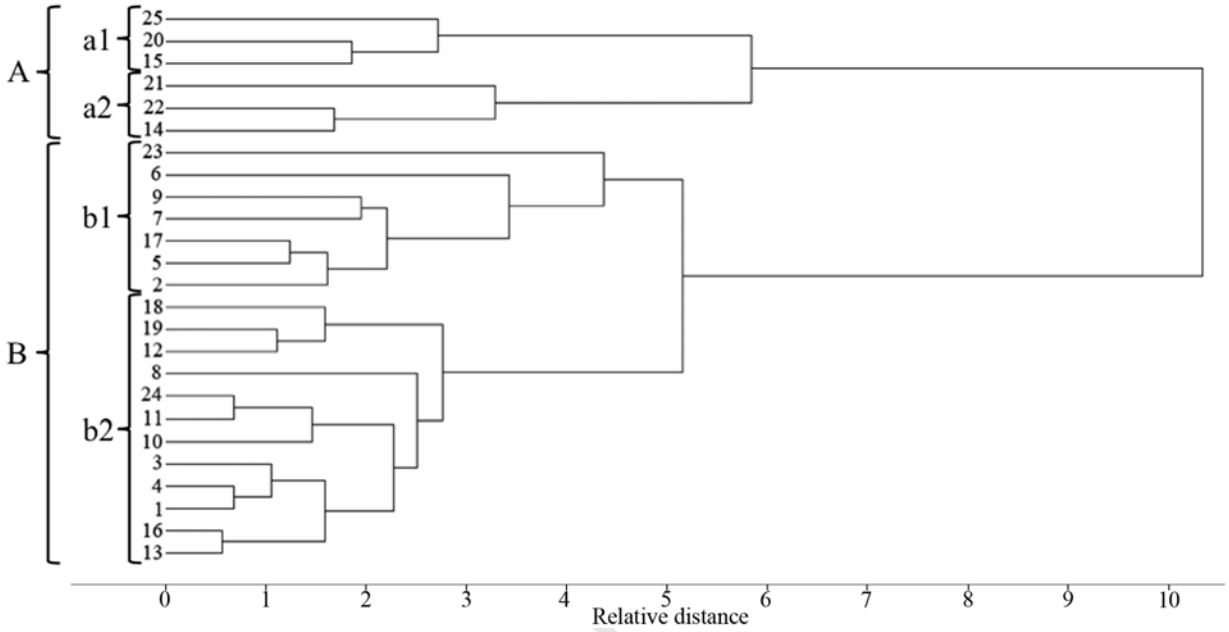


Figure 7: Chromatogram examples of the four donor sub-groups identified through hierarchical cluster analysis. 1-decanol is the internal standard and its concentration is identical in the four chromatograms.

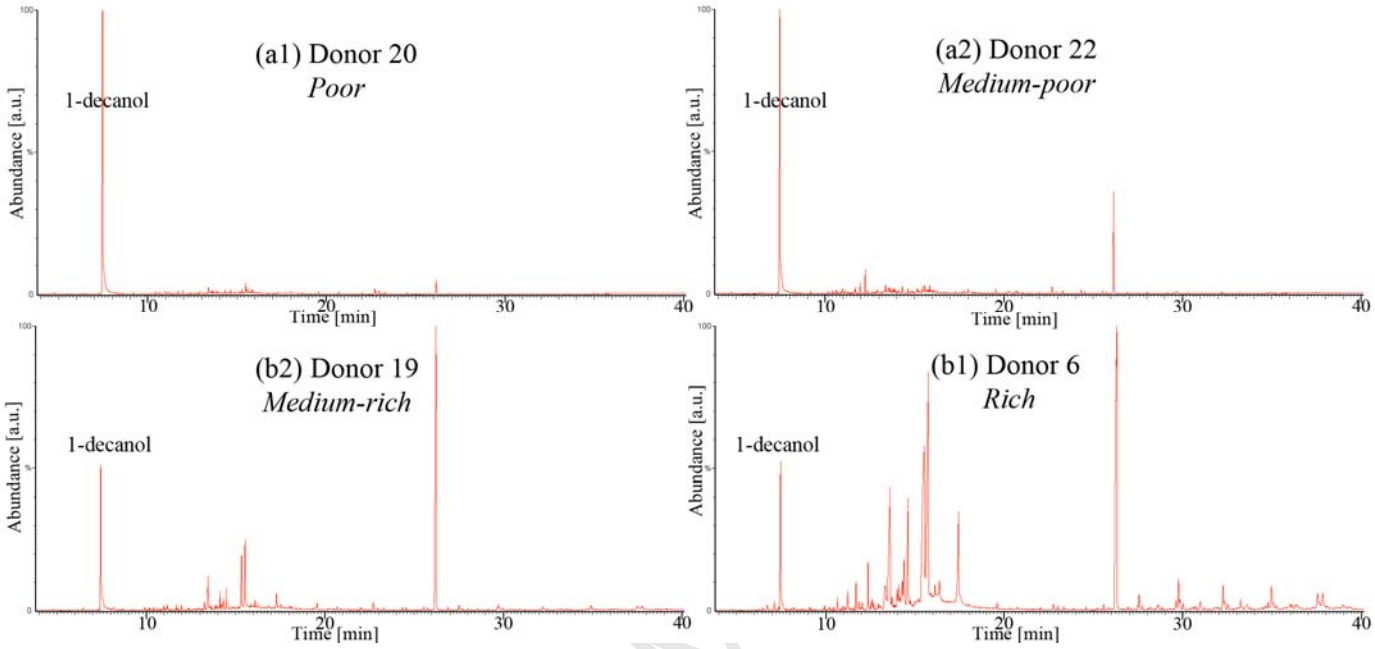


Figure 8: Classification of donors 1, 2, 4-18, 20-15 and replicates of donor 3 (17 replicates) and donor 19 (19 replicates) using hierarchical cluster analysis with Euclidean distance and complete linkage clustering. The normalisation to sum followed by the logarithm (NormSum+LOG) was used. Two major clusters are clearly visible: (A) donors 14, 15, 20, 21, 22 and 25 as well as three replicates of donor 3 and two replicates of donor 19 and (B) donors 1, 2, 4-13, 16-18, 23 and 24 as well as 14 replicates of donor 3 and 17 replicates of donor 19. Highlighted in red dark are the wrong-classified replicates and in light green the correct-classified replicates when considering main groups (A) and (B).

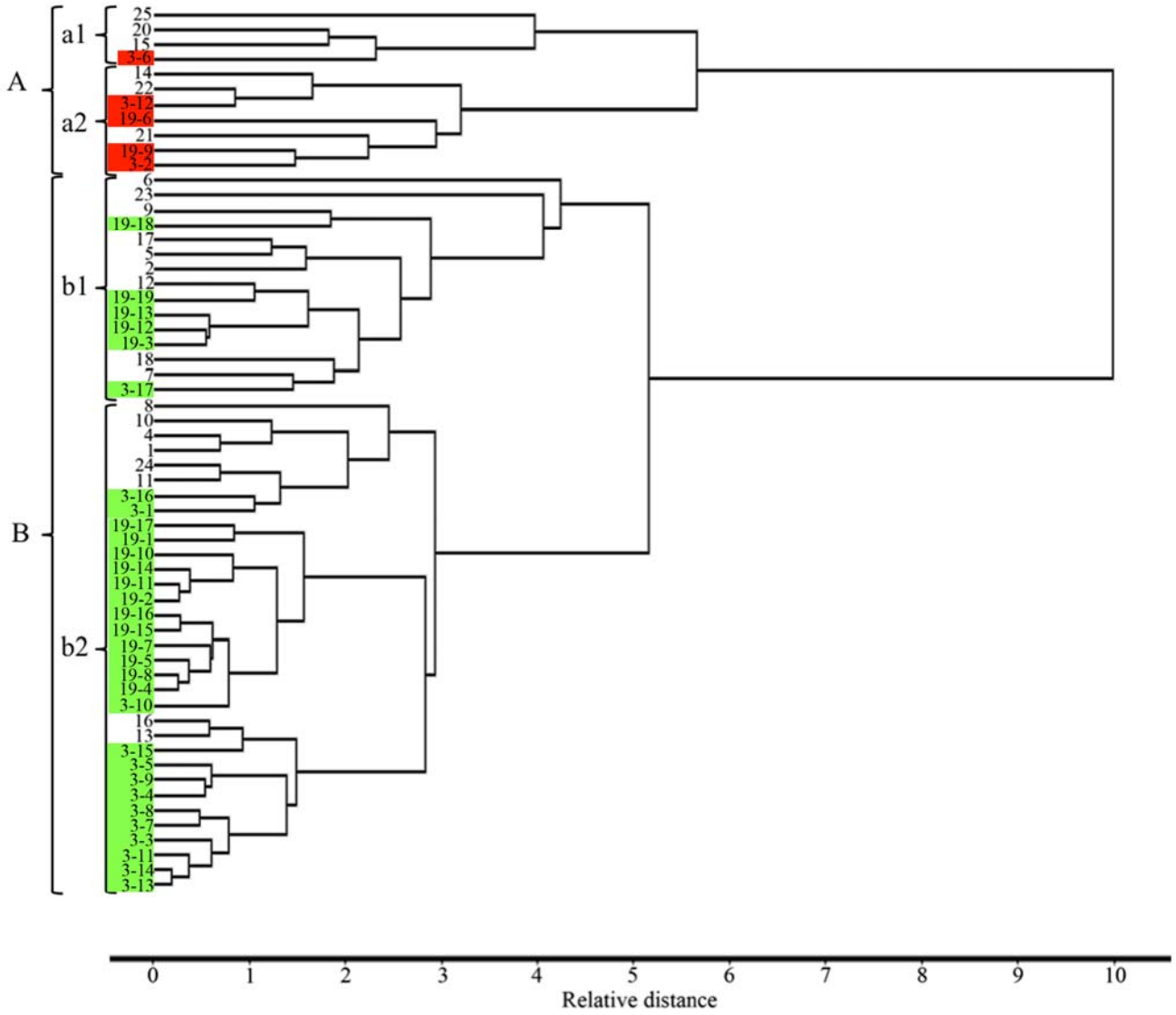


Table 1: Characteristics of the fingermark donors
Origin: C = Caucasian, A = Asian
Diet: O = omnivore, V = vegetarian
** Genetic cholesterol level higher than the norm, possible hypercholesterolemia (based on last medical control)*

No.	Sex	Age	Origin	Smoke	Diet	Metabolic disease	Cosmetics
1	F	25	C	No	O	No	Face powder
2	F	26	C	No	O	No	Foundation crème
3	M	25	C	No	O	No	Face crème
4	F	25	C	No	O	No	No
5	F	27	C	Yes	O	No	Hair gel
6	M	33	C	No	O	No	Hair gel, perfume
7	M	26	C	Yes	O	Acne	Face crème
8	M	27	C	No	V	No	No
9	M	25	C	No	O	No	Hair wax, perfume
10	M	26	C	No	O	No	Hair gel
11	F	32	C+A	No	O	No	Face crème
12	F	31	C	No	O	No	No
13	M	26	C	No	O	No	No
14	M	26	C	No	O	No	No
15	M	38	C	No	O	No	No
16	M	38	C	No	O	No	No
17	F	33	C	No	O	Hyperchol.*	Face crème
18	F	57	C	Yes	O	No	No
19	F	24	C	No	O	No	No
20	M	47	C	No	O	No	No
21	F	46	C	No	O	Hyperchol. Hypothyroid.	Face crème
22	F	26	C	No	O	No	No
23	F	34	C	Yes	O	No	Face crème
24	M	26	C	No	O	No	No
25	F	32	C	No	O	No	No

Table 2: List of the 104 compounds detected in the fingermarks of 25 donors**.** The **bold** compounds were reported for the first time as endogenous constituents of fingermark residue, while the10 highlighted compounds were selected as target compounds for the evaluation of the variability and the development of the classification model. The following abbreviations and indications were used:
***Rt**: retention time*
***MW**: molecular weight*
***FA**: fatty acids*
***WE**: wax esters*
***ST**: sterol*
***STprec**: ST precursor*
***OX**: oxidation products*
***VIT**: vitamin*
***MS**: analysis of mass spectra*
***DB**: comparison with a mass spectra library*
***STD**: comparison with a standard*
***TTT**: 5,9,13-trimethyl-tetradeca-4,8,12-triene-al*
***TOP**: 4,8,13,17,21-tetra-methyl-octadeca-4,8,12,16,20-pentaene-al*
***TOT:** 4,9,13,17-tetramethyl-octadeca-4,8,12,16-tetraene-al*
***GA**: geranyl acetone*
***WE (A:B, C:D)**: **A**: # of carbons in fatty alcohol, **B**: # of double bonds in fatty alcohol, **C**: # of carbons in fatty acid, **D**: # of double bonds in fatty acid.*
***** Compound only present in one fingermark over the two fingermarks of **one** donor or ****** of **two** donors*

Rt (min)	Compounds names (alternative names)	MW	Target (qualifiers)	Type	ID	# donors n=25
5.63	Octanoic acid (caprylic acid)	144	60	FA	MS+DB	22
7.2	Nonanoic acid (pelargic acid)	158	60	FA	MS+DB	25
8.52	Decanoic acid (capric acid)	172	60	FA	MS+DB	25
9.774	**Squalene oxidation product (GA)**	**194**	**69**	**SQUAL OX**	**MS+DB**	**25**
11.008	Dodecanoic acid (lauric acid)	200	60	FA	MS+DB	25*
11.822	**Isopropyl dodecanoate (isopropyl laurate)**	**242**	**60 (102, 201)**	**WE**	**MS+DB**	**25**
12.155	Tridecanoic acid	214	73	FA	MS+DB	25*
13.082	Tetradecenoic acid (myristoleic acid)	226	55	FA	MS+DB	25
13.269	Tetradecanoic acid (myristic acid)	228	73	FA	DB+STD	25
13.989	**Isopropyl tetradecanoate (isopropyl myristate)**	**270**	**60 (102)**	**WE**	**DB**	**25**
14.049	**Isopropyl-12-methyltetradecanoate**	**285**	**57 (185)**	**WE**	**DB**	**12**
14.129	Pentadecenoic acid	240	55	FA	MS+DB	22
14.189	**Squalene oxidation product (TTT)**	**248**	**69**	**SQUAL OX**	**MS+DB**	**25**
14.316	Pentadecanoic acid	242	73	FA	DB+STD	22
15.143	Hexadecenoic acid (palmitoleic acid)	254	55	FA	MS+DB	25
15.336	Hexadecanoic acid (palmitic acid)	256	73	FA	DB+STD	25
15.963	**Isopropyl hexadecanoate (isopropyl palmitate)**	**298**	**60 (102)**	**WE**	**MS+DB**	**23**
17.117	Octadecenoic acid (oleic acid)	282	55	FA	MS+DB	25
17.377	Octadecanoic acid (stearic acid)	284	73	FA	DB+STD	25
17.99	**Pentyl benzoate (benzoic acid pentyl ester)**	**192**	**105**	**Benz. ac. ester**	**MS+DB**	**15**
19.377	**Hexyl benzoate (benzoic acid hexyl ester)**	**206**	**105**	**Benz. ac. ester**	**MS+DB**	**15**
19.437	**Squalene oxidation product (TOP)**	**316**	**69**	**SQUAL OX**	**MS+DB**	**25**
20.771	**Heptyl benzoate (benzoic acid heptyl ester)**	**220**	**105**	**Benz. ac. ester**	**MS+DB**	**13***
21.951	Unknown WE	-	112 (239, 256)	WE	MS	16**
22.118	**Octyl benzoate (benzoic acid octyl ester)**	**234**	**105**	**Benz. ac. ester**	**MS+DB**	**12****
23.732	**WE (10:0, 11:0)**	**326**	**187**	**WE**	**MS**	**11***
24.485	Unknown WE	-	112 (267, 284)	WE	MS	12**
25.266	**WE (14:0, 12:0)**	**396**	**201**	**WE**	**MS**	**15***
25.299	WE (12:0, 15:0)	410	243	WE	MS	18
25.419	**Squalene oxidation product (TOT)**	**384**	**69**	**SQUAL OX**	**MS+DB**	**25**
25.766	**WE (14:0, 13:0)**	**410**	**215**	**WE**	**MS**	**11**

26.053	Squalene (SQUAL)	410	69	STprec	DB+STD	25
26.933	**Cholesterol ester (Cholest-5-en-3-ol-propanoate)**	**-**	**145 (368, 386)**	**ST**	**MS**	**22**
27.146	WE (14:0, 14:0)	424	229	WE	MS	5
27.32	WE (12:0, 16:1)	422	236	WE	MS	19
27.386	Myristyl myristoleate (14:0, 14:1)	422	208	WE	MS+STD	25
27.47	Possible squalene oxidation product	-	69	SQUAL OX	MS	9
27.53	Possible squalene oxidation product	-	69	SQUAL OX	MS	6
27.573	Myristyle myristate (14:0, 14:0)	424	229	WE	MS+STD	22
28.053	**WE (14:0, 15:1)**	**436**	**222**	**WE**	**MS**	**24**
28.26	WE (14:0, 15:0)	438	243	WE	MS	21*
28.407	WE (14:0, 15:0)	438	243	WE	MS	20
28.467	**WE (14:0, 15:1)**	**436**	**222**	**WE**	**MS**	**22**
28.6	**Gamma – tocopherol (vitamin E)**	**416**	**151 (416)**	**VIT**	**DB**	**2**
28.68	WE (14:0, 15:0)	438	243	WE	MS	21
28.960	WE (13:0, 16:1)	436	236	WE	MS	11
29.15	WE (14:0, 16:1)	450	236	WE	MS	9
29.447	Cholesterol (CHOL)	386	145 (368, 386)	ST	DB+STD	25
29.594	Myristyl palmitoleate (14:0, 16:1)	450	236	WE	MS+STD	25
29.667	Palmityl myristoleate (16:0, 14:1)	450	208	WE	MS+STD	22*
29.854	Myristyl palmitate (14:0, 16:0)	452	257	WE	MS+STD	25
30.307	WE (14:0, 17:1)	646	250	WE	MS	6
30.394	WE (16:0, 15:1)	464	222	WE	MS	12*
30.454	**WE (14:0, 17:0)**	**466**	**271**	**WE**	**MS**	**20**
30.581	WE (17:0, 14:1)	464	208	WE	MS	22
30.814	WE (15:0, 16:1)	464	236	WE	MS	22
31.087	**WE (15:0, 16:0)**	**466**	**257**	**WE**	**MS**	**20**
31.168	WE (16:0, 16:0)	480	257	WE	MS	6
31.328	**WE (17:0, 15:1)**	**478**	**222**	**WE**	**MS**	**8**
31.608	WE (16:0, 16:1)	478	236	WE	MS	12*
31.634	**Cholesterol intermediate (lanost-8-en-3β-ol)**	**428**	**395**	**ST**	**MS+DB**	**9**
31.794	**WE (17:0, 15:1)**	**478**	**222**	**WE**	**MS**	**19**
31.868	WE (16:0, 16:0)	480	257	WE	MS	17
32.088	Palmityl palmitoleate (16:0, 16:1)	478	236	WE	MS+STD	25
32.181	**WE (18:0, 14:1)**	**478**	**208**	**WE**	**MS**	**22**
32.348	Palmityl palmitate (16:0, 16:0)	480	257	WE	MS+STD	25
32.394	WE (18:0, 14:0)	480	229	WE	MS	18
32.58	WE (17:0, 16:1)	492	236	WE	MS	10*
32.901	WE (17:0, 16:1)	492	236	WE	MS	19
32.968	**WE (18:0, 15:1)**	**492**	**222**	**WE**	**MS**	**3**
33.088	WE (major 17:0, 16:1)	492	236	WE	MS	25
33.168	**WE (17:0, 16:0)**	**494**	**257**	**WE**	**MS**	**22****
33.421	WE (16:0,16:1)	492	236	WE	MS	23
33.695	**WE (18:0, 15:0)**	**494**	**243**	**WE**	**MS**	**17**
33.768	WE (18:0, 16:1)	506	236	WE	MS	3
33.988	**WE (19:0, 15:1)**	**506**	**222**	**WE**	**MS**	**2**
34.075	**WE (20:0, 14:0)**	**508**	**229**	**WE**	**MS**	**7**
34.262	WE (18:0, 16:1)	506	236	WE	MS	11*
34.388	**WE (20:0, 14:1)**	**506**	**208**	**WE**	**MS**	**21**
34.495	WE (18:0, 16:1)	506	236	WE	MS	20
34.602	**WE (20:0, 14:0)**	**508**	**229**	**WE**	**MS**	**22**
34.802	Stearyl palmitoleate (18:0, 16:1)	506	236	WE	MS+STD	25
34.902	**WE (20:0, 14:1)**	**506**	**208**	**WE**	**MS**	**22**
35.075	Stearyl palmitate (18:0, 16:0)	508	257	WE	MS+STD	23
35.442	WE (20:0, 15:0)	522	243	WE	MS	9

35.669	**WE (19:0, 16:1)**	**520**	**236**	**WE**	**MS**	**10**
35.862	WE (20:0, 16:1)	534	236	WE	MS	1
35.969	**WE (21:0, 15:0)**	**536**	**243**	**WE**	**MS**	**24**
36.216	WE (20:0, 15:0)	522	243	WE	MS	19
36.46	WE (20:0, 15:0)	522	243	WE	MS	7
36.582	WE (20:0, 16:1)	534	236	WE	MS	1
36.836	WE (20:0, 16:1)	534	236	WE	MS	7
37.122	WE (20:0, 16:1)	534	236	WE	MS	8
37.363	WE (20:0, 16:1)	534	236	WE	MS	24
37.46	**WE (22;0, 14:0)**	**536**	**229**	**WE**	**MS**	**22**
37.663	WE (major 20:0, 16:1)	534	236	WE	MS	25
37.923	**WE (20:0, 16:0)**	**536**	**257**	**WE**	**MS**	**19**
38.236	WE (20:0, 16:1)	534	236	WE	MS	5
38.43	**WE (21:0, 16:1)**	**548**	**236**	**WE**	**MS**	**13***
38.59	**WE (21:0, 16:1)**	**548**	**236**	**WE**	**MS**	**8**
38.79	**WE (21:0, 16:1)**	**548**	**236**	**WE**	**MS**	**20**
39.17	**WE (21:0, 16:1)**	**548**	**236**	**WE**	**MS**	**18**
39.503	**WE (21:0, 16:0)**	**550**	**257**	**WE**	**MS**	**3**
39.616	**WE (21:0, 16:1)**	**548**	**236**	**WE**	**MS**	**1**

Table 3: Quantification of palmitic acid (PALM), squalene (SQUAL), cholesterol (CHOL), myristyl myristate (MM) and myristyl palmitoleate (MPO) in the fingermarks of 25 donors, expressed in ng/FM.
S_{x0}*: standard deviation of the concentration,* ***LoD****: limit of detection,* ***LoQ****: limit of quantification.*

	Minimum	**± S_{x0}**	**Maximum**	**± S_{x0}**	**LoD**	**LoQ**
PALM	169.19	± 45.93	13'761.86	± 1'766.51	45.89	152.97
SQUAL	78.76	± 16.46	5'662.56	± 377.42	16.95	56.50
CHOL	76.71	± 19.22	978.17	± 184.17	20.07	66.90
MM	4.33	± 2.00	70.62	± 8.17	1.25	4.15
MPo	6.56	± 2.86	1023.97	± 108.55	1.71	5.70

Table 4: Summary and comparison of the classification obtained in the dendrograms of Figures 6 and 8. Highlighted in dark red is the number of wrongly-classified replicates (5) and in light green the number of correctly-classified replicates (31) into the main groups (A) and (B). Donors 12 and 18 (**bold***) were classified into different sub-groups when dendrograms of Figures 6 and 8 were compared.

			Figure 6	**Figure 8**		
			Donors No.	**Donors No.**	**# replicates of D3 (n = 17)**	**# replicates of D19 (n = 19)**
Groups	**A**	**a1**	15, 20, 25	15, 20, 25	1	/
		a2	14, 21, 22	14, 21, 22	2	2
	B	**b1**	2, 5, 6, 7, 9, 17, 23	2, 5, 6, 7, 9, **12***, 17, **18***, 23	1	5
		b2	1, 3, 4, 8, 10, 11, **12***, 13, 16, **18***, 19, 24	1, 4, 8, 10, 11, 13, 16, 24	13	12

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