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A comparison of the natural and groomed fingermark lipid composition of different donors using GC/MS

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ABSTRACT

The lipid composition of natural fingermarks was studied and compared with the composition of groomed residue. Approximately 100 specimens were collected from 6 donors over three sessions (in October, December and July) and analysed using gas chromatography / mass spectrometry (GC/MS). The measured lipid content was generally lower and more variable in natural fingermarks than in groomed fingermarks. Some significant variability was noticed. Relative standard deviations were the highest between donors (generally above 100%) but were also relatively high within donor within a session (from 21% to 80%) and between sessions (from 34% to 126%). The fingermarks from one of the donors generally contained higher relative amounts of lipids in both groomed and natural residue compared to the others. All other fingermarks led to very variable amounts and did not allow classifying the other donors as constantly "good" or "poor" donors. Squalene was the major compound in all marks, particularly in groomed specimens. A correlation between squalene, cholesterol, myristic acid, palmitoleic acid, stearyl palmitoleate and pentadecanoic acid was highlighted. Oleic and stearic were also correlated together but generally more in natural than groomed marks. The obtained results may be particularly useful to better understand the detection mechanisms for techniques targeting lipids and to develop artificial fingermark secretions to further support the development of detection techniques.

1. Introduction

Thanks to their highly variable ridge pattern, fingermarks are pivotal to investigating crimes, particularly for identification purposes. While fingermarks can be visible to the naked eye, many remain latent and need to be visualized using optical, physical or chemical processes[1,2]. The residues present in fingermarks are the targets of the different techniques used to detect them [3–5].

Fingermark residues are composed of a complex mixture of eccrine sweat, sebum, and exogenous compounds transferred by contact with the fingertips. Eccrine sweat originates from the eccrine glands and is mainly composed of water [6], the remaining constituents including sodium chloride, potassium, ammonia, urea, amino acids, sugars, vitamins, and proteins [7]. These molecules are secreted by sweat pores disposed over the whole body, including the ridge skin area. Sebum originates from sebaceous glands, and is mostly composed of lipids such as glycerides, fatty acids, wax esters and sterols [8]. Sebum glands are found all over the body except on the hands and feet. Thus, lipids are transferred on the fingertips by secondary contacts, for example, when the fingers touch the face or the hair. Compounds from the apocrine sweat, whose glands are found in the genital, breast, inguinal and axillary regions, can occasionally be present in fingermarks but were rarely studied [4]. Finally, molecules from exogenous sources such as food, cosmetics or drugs can also be found in fingermarks by contact with the fingertips [9,10].

Lipids are an essential part of the fingermark residue and play a role in several detection methods, such as dry powders, Oil Red (ORO), Nil Red (NR) and physical developer (PD) [11–14]. In the case of PD, the role of lipids is still unclear. They may actively participate to the detection of fingermarks, while also act as entrapment matrix for compounds of eccrine origin [15–17]. Therefore, studies aiming to increase the knowledge about fingermark lipid composition are important to better understand and optimise detection methods. Additionally, recent endeavours to develop artificial secretions to

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Table 1
- Summary of fingermarks deposition and storage conditions. In total, 102 fingermarks were collected and 101 analysed in this study, because one donor was missing at the October session and one result was lost due to instrumental issue.

# Donors	6
# Session	October 2018 December 2018 July 2019
# Fingermarks	6 per donor (3 natural and 3 groomed)
Weight reported on the scale	500 g + /- 100 g
Duration of deposition	10 s
Subtract	A4 canon office paper Black Label Zero
Storage	24 h in a plastic container

Table 3
- Averaged quantities of Squalene (SQUAL), Palmitic acid (PALM), Cholesterol (CHOL), Myristyl myristate (MM) and Myristyl palmitoleate (MPo) in nanogram per fingermark (ng/FM). A total of natural (n = 50) and groomed (n = 51) fingermarks have been analysed from 6 donors over 3 sessions.

	NATURAL		GROOMED		
Lipids	ng/FM	RSD	ng/FM	RSD	LOQ
SQUAL	556 ± 172	31%	2315 ± 532	23%	8,4
PALM	438 ± 153	35%	700 ± 175	25%	300
CHOL	345 ± 79	23%	749 ± 135	18%	480
MM	73 ± 8	11%	111 ± 6	5%	50
MPo	69 ± 6	9%	104 ± 10	10%	60
TOTAL	**1481 ± 418**	**28%**	**3979 ± 858**	**22%**	-

test detection methods also benefit from fingermark composition studies [8,18,19].

Several studies have already focused on the lipid composition of fingermarks [20–24], mainly using gas chromatography (GC) and liquid chromatography (LC) coupled to mass spectrometry (MS), but also other mass spectrometric methods [5,23,25–32]. As lipids are secreted by the sebaceous glands, most studies were carried out using groomed fingermarks (i.e., marks obtained by rubbing the fingertips on the donor's face or neck to enrich the fingertips in sebum). These studies identified over 200 compounds in the fingermarks, including squalene, cholesterol, over 10 fatty acids and 70 wax esters [5], and over 100 di- and triglycerides [30,31]. More than 35 compounds were detected in the fingermarks of all the donors (25 donors in [5] and 10 in [31]) and may thus be interesting targets for the development of detection methods.

While the qualitative composition of fingermarks is relatively similar within and between donors, it was observed that the absolute and relative lipid quantities vary significantly from person to person (i.e., inter-variability) [5,21,25,26,33]. These variations were used to propose a donor classification model to differentiate good vs poor lipid donors [5,25], or to predict gender and smoking habits [20]. However, significant variations were also observed within the fingermarks of the same person (i.e., intra-variability) [5,23,25,26,33,34] even when the fingermarks were deposited the same day. After deposition, the ageing of fingermarks also influences the composition, for example, through a decrease of compounds such as squalene and cholesterol [22,30,32,33,35–38].

Very few studies studied the natural fingermark composition [20–23,38,39]. Natural fingermarks are composed of the residue naturally found on the fingertips when hands are neither deliberately washed nor groomed before deposition [40,41]. They better represent the fingermark composition as found at a crime scene or on an exhibit relevant to an investigation [8]. Two previous studies compared the composition of natural and groomed fingermarks [20,23]. Groomed marks contained significantly more lipids (i.e., squalene and fatty acids), while the amino acid content was not quantitatively influenced by the grooming process.

This work aimed at further studying the lipid composition of natural fingermarks, compared to groomed fingermarks, both qualitatively (which lipids?) and quantitatively (in which relative quantity?). Based on the classification proposed in previous studies [5,25]: 3 good, 2 intermediate and 1 poor donors were selected to produce natural and groomed fingermarks over three sessions. 17 lipids, present in the fingermarks of all donors [5] were studied using GC/MS. Results obtained for natural and groomed fingermarks were compared and discussed. Correlations between compounds

Table 2
– List of compounds detected in the fingermarks with a summary of their known primary sources. Most of the compounds found in the sebum can also be found in skin products and found in other sources such as food [5,9,25].

Compound	RT (min)	Type	Main known sources
Pelargic (nonanoic) acid	7,2	Fatty acid	Synthetized by plants, used as a food additive and to prepare plasticizers and lacquers[45,46]
Capric (decanoic) acid	8,5	Fatty acid	Abundant in coconut and almond oil, also found in animal fat and milk[45,46]
Lauric (dodecanoic) acid	10,9	Fatty acid	Very abundant in coconut and palm fat, also found in animal milk, primarily used in cosmetics as surfactant and preservative[29,46–48]
Myristic (tetradecanoic) acid	13,2	Fatty acid	Very abundant in nutmegs, palm and coconut oil, also found in animal fat and milk[46–48]
Pentadecanoic acid	14,2	Fatty acid	1,2% of butterfat from cow milk, traces in some plants and fishes[49–51]
Palmitoleic (hexadecenoic) acid	15,1	Fatty acid	It is a common constituent of human adipose tissue. It is biosynthesized from palmitic acid by the action of the enzyme Stearoyl-CoA. Natural occurrence in macadamia nuts and other vegetal oils[49,52–54]
Palmitic (hexadecenoic) acid*	15,3	Fatty acid	Very abundant in meat, cheese, and milk from animals, also found in palm oil, used in cosmetics as cream and soap component[47,49,51–57]
Isopropyl palmitate	15,9	Fatty acid ester	Synthetized from the ester of isopropyl alcohol and palmitic acid, used as an emollient, moisturizer, and anti-static agent[5,26,47]
Oleic (octadecenoic) acid	17,3	Fatty acid	Very abundant in vegetables as olive oil and found in some animals[47,48,54,58]
Stearic (octadecanoic) acid	17,4	Fatty acid	Very abundant in animal fat, also found in palm oil and shea oil, used in cosmetics as surfactant agent [47,49,51–55,57]
Squalene*	25,9	Sterol	Synthesized by all plants and animals as a biochemical intermediate and precursor of sterols -such as cholesterol[27,28,32,46–49,51,52,54–57,59–64]
Myristyl myristate*	27,4	Wax ester	Synthetized from myristic acid by cosmetic industries, primarily used in various cosmetics and skincare as an emollient, texture enhancer and emulsifier[5,26,47]
Cholesterol*	29,2	Sterol	Synthesized by all animals[27,28,32,46,47,50–53,55,57,58,60,65]
Myristyl palmitoleate*	29,6	Wax ester	Wax ester of palmitic acid, emollient in cosmetic[5,26,47]
Myristyl palmitate	29,8	Wax ester	Wax ester from the condensation of palmitic acid in animal and vegetal sources, emollient in cosmetic [5,26,47]
Palmityl palmitate	32,2	Wax ester	Resulting from the condensation of palmitic acid with palmityl alcohol, used as a thickener and emollient in cosmetic [5,26,47]
Stearyl palmitoleate	34,5	Wax ester	Derived from stearyl alcohol and palmitic acid, used in cosmetics as shampoo additive[5,26,47]

RT = retention time (in minutes). Quantified compounds are indicated with a star* .

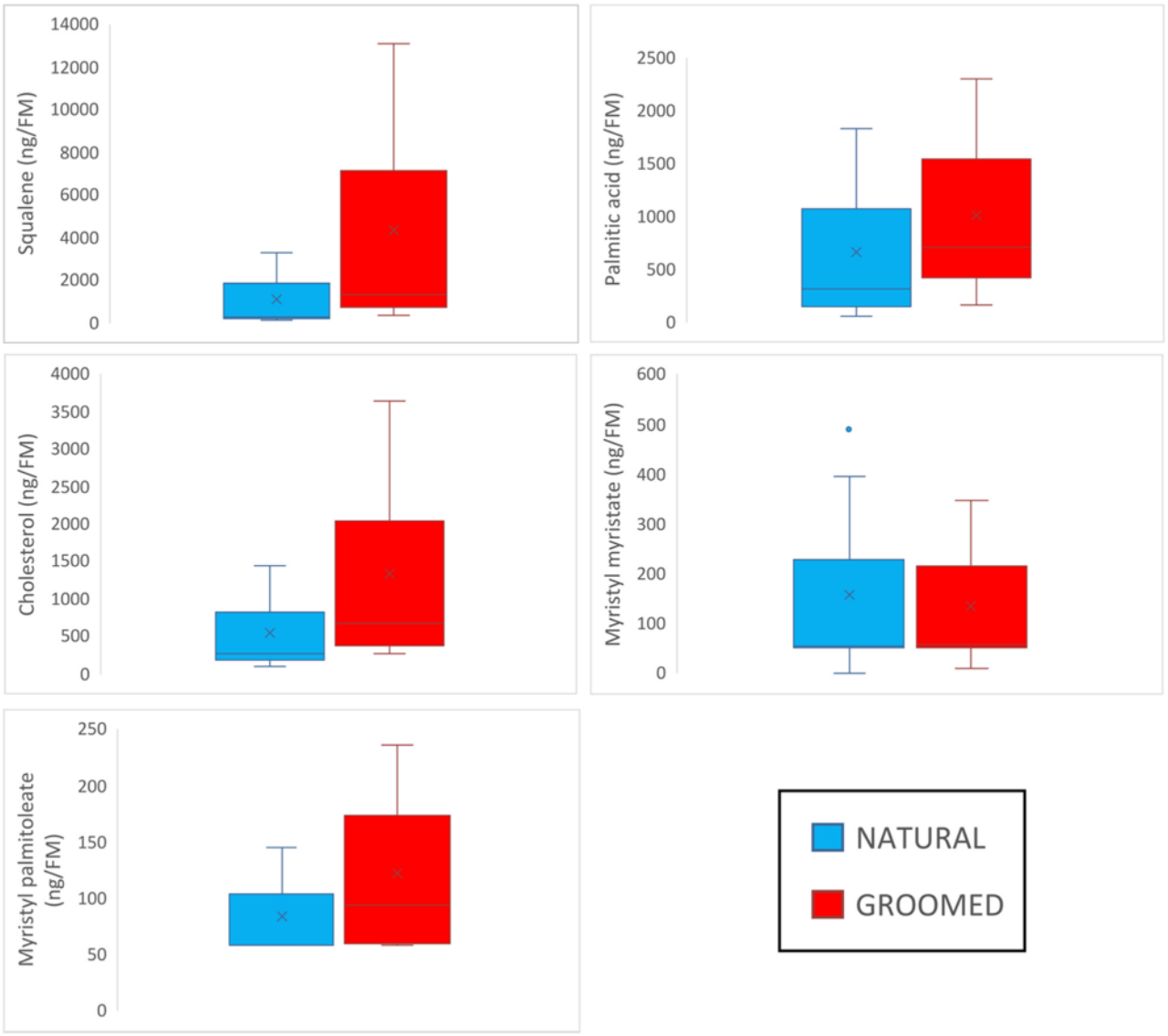

Fig. 1. - Distribution of the measured quantities of squalene, palmitic acid, cholesterol, myristyl myristate and myristyl palmitoleate in groomed and natural fingermarks. Results are represented in nanograms per fingermark, natural (n = 50) and groom (n = 51). Whiskers from boxplots show the distribution from the minimum to the maximum value. Box percentiles are from 1% to 99%, the average is indicated with the x inside the box, and the median with a horizontal line within the box.

were investigated, and the stability of donor classification was tested over three deposition sessions, both for natural and groomed marks.

2. Materials and methods

2.1. Fingermark collection

Fingermarks were collected from 6 donors. A preliminary study aimed at selecting donors based on different relative lipid abundance. Donors were also chosen based on their availability during the experiment (3 collection sessions over 10 months). Donors were informed about the procedure filled in a consent form and a questionnaire before each session. Collected data were anonymized in compliance with ethical guidelines.[5]

The guidelines from the International Fingermark Research Group (IFRG) [42] were followed. Fingermarks were deposited on A4 paper White office paper CANON 211 Black Label zero 80 mg/m^2. Donors followed their tasks usually before deposition but were instructed to avoid handwashing with soap for at least one hour prior to deposition. In addition, donors were asked to rub the fingers of both hands together to homogenize residue before collection.

Six marks were collected per donor at each session (index, middle and ring fingers). Three natural marks were first collected per donor. Then, the same fingers were rubbed on the forehead, neck and the edge of the nose to collect three groomed fingermarks per donor during the same session. Fingermarks were collected during three different sessions in the following order over 10 months: in October 2018 (average outside temperature 11 °C), December 2018 (average outside temperature 6 °C) and July 2019 (average outside temperature 25 °C).[6] The temperature in

[5] The regional ethical commission (Commission cantonale VD d'éthique de la recherche sur l'être humain) checked and approved the developed procedures (protocol 2017–00265). https://www.cer-vd.ch/ (last access: October 2021).

[6] The average outside temperature was measured by www.meteosuisse.admin.ch (last access: October 2021).

Table 4
- Mean normalised peak areas (NPA) and RSD% of 17 target lipids measured in natural and groomed fingermarks. *The mean NPA values were multiplied by 1000 for a better legibility.*

	NATURAL (n = 50)		GROOMED (n = 51)	
Lipids	NPA	RSD%	NPA	RSD%
Pelargic acid	7,7	75%	9,6	48%
Capric acid	1,4	62%	2,2	67%
Lauric acid	1,4	131%	4,7	156%
Myristic acid	3,0	150%	10,9	149%
Pentadecanoic acid	1,4	176%	5,6	165%
Palmitoleic acid	7,0	217%	44,4	227%
Palmitic acid	10,3	88%	17,2	56%
Isopropyl palmitate	2,0	118%	3,2	149%
Oleic acid	4,3	98%	5,2	64%
Stearic acid	3,9	122%	4,2	81%
Squalene	731,4	137%	3104,3	101%
Myristyl myristate	7,3	266%	12,0	157%
Cholesterol	7,2	99%	18,7	85%
Myristyl palmitoleate	0,9	165%	3,6	100%
Myristyl palmitate	1,7	101%	6,3	96%
Palmityl palmitate	1,1	112%	6,1	111%
Stearyl palmitoleate	2,0	161%	9,2	107%
AVERAGE (all lipids)	**46,7**	**134%**	**192,2**	**113%**

the office is not air-conditioned, but a heating system is switch-on when the outside temperature is low. In total, 102 fingermarks were collected and 101 results were obtained due to instrumental issues (see details in Table 1).

The deposition time and pressure were monitored by putting the paper substrate on a kitchen scale and asking the participants to press on the paper for 10 s at 500 ± 100 g. During the deposition, the contour of the fingers was drawn with a pencil to subsequently locate the marks on the paper substrate. All fingermarks were stored in a plastic container in a cupboard for 24 h before being extracted (see Table 1).

2.2. Sample extraction

Fingermarks on paper were introduced in cap glass vials (12 ×32 mm vials, purchased from Interchim) filled with 1.5 mL of dichloromethane (99.99% Sigma Aldrich) for 1 min and slightly agitated with a vortex for 10 s. All the liquid was then transferred using a glass pipette to another vial and evaporated to dryness to concentrate the extracted residue under a stream of nitrogen. The residue was then re-dissolved in 20 µl of dichloromethane containing an internal standard (0.05 mg/mL of 1-decanol, purchased from Sigma Aldrich) and transferred in a 110 µl conical glass insert (purchased from BGB Analytik) for injection in the GC/MS instrument.

2.3. GC-MS analysis

The samples were analysed using a gas chromatograph coupled with a mass spectrometer from Agilent (7000 Series Triple Quad GC/MS 7890 A Series). The column used for the analysis was an HP5- MS (30 m x 0.25 mm × 0.25 mm). The carrier gas was helium with a constant flow of 1 mL/min. 1 µl was injected in splitless mode with a purge time of 1.5 min from every sample. The injector was 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 a final isothermic step at 320 °C during 2 min. A solvent delay of 3.6 min was applied, and the transfer line temperature was maintained at 300 °C. The mass analyser used was a quadrupole at 150 °C and set in scan mode between 40 and 550 *m/z*.

Palmitic acid, squalene, cholesterol, myristyl myristate and myristyl palmitoleate standards were purchased for quantification purposes from Sigma-Aldrich (fatty acids and sterols) and Nu-Chek Prep (wax esters). They were chosen to represent the main lipid types identified in this work (see Table 2) and were detected in all fingermarks [5]. Calibration was performed using standard solutions of the five lipids at the following concentrations: 100, 80, 60, 40, 20, 10, 7, 5, 3 and 1 µg/ mL. The peak areas of the following target ions (TI) were used for quantification: T1 = 129 *m/z*, for palmitic acid, TI = 145 *m/z* for cholesterol, TI = 69 *m/z* for squalene, TI = 229 *m/z* for myristyl myristate and TI = 236 *m/z* for myristyl palmitoleate. An internal standard (1-decanol, 0.05 mg/mL, TI= 55 *m/z*) was used to normalise the peak areas. The limits of detection (LoD) and

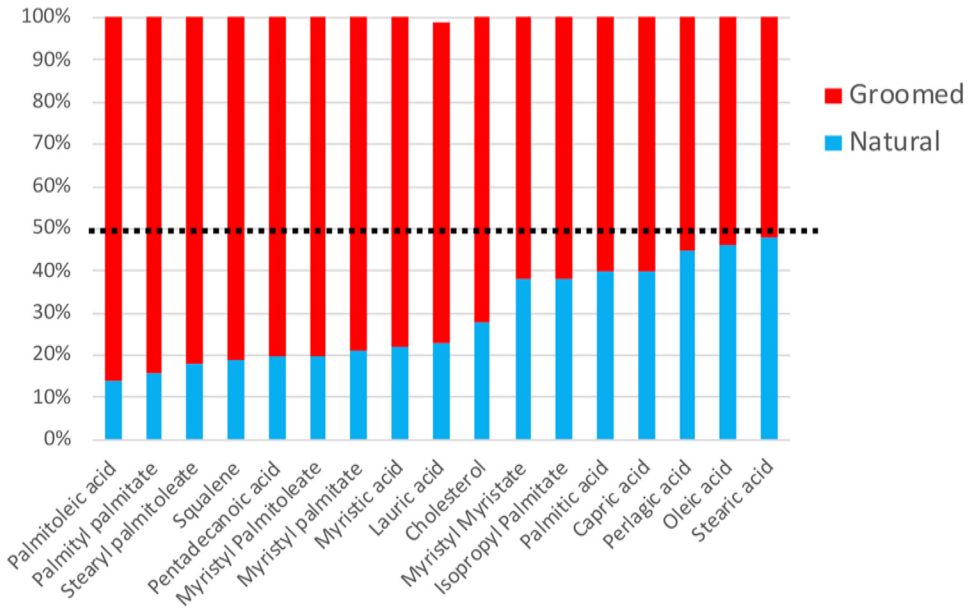

Fig. 2. - Average lipid proportions between natural (n = 50) and groomed (n = 51) fingermarks collected from 6 donors.

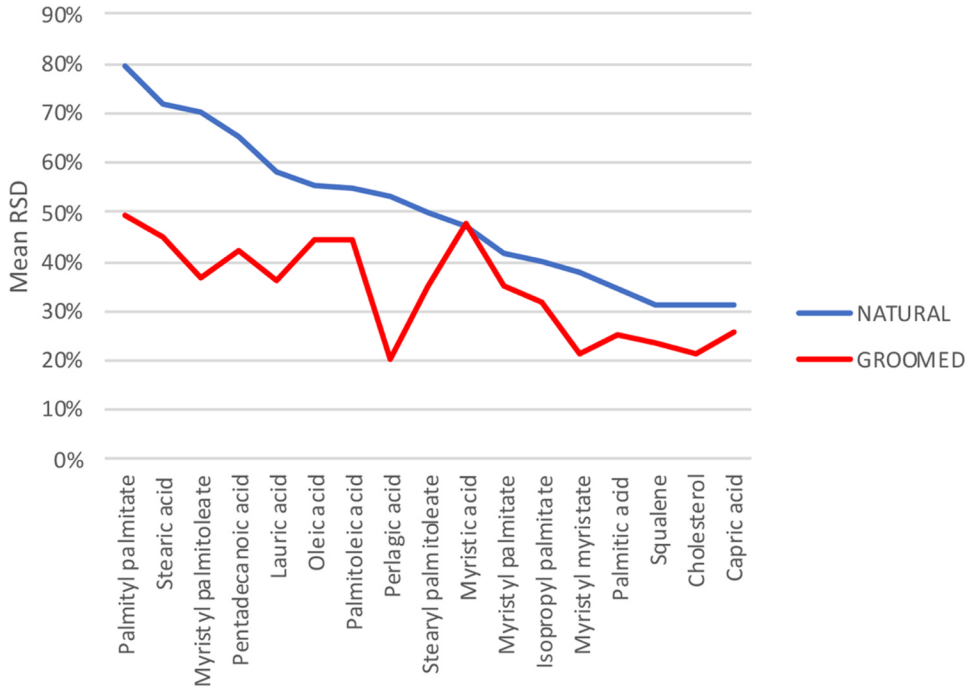

Fig. 3. - Comparison of the average relative standard deviation (RSD) of the 17 target compounds. The RSD values were calculated from the results obtained for three fingermarks collected per donor and sessions for natural specimens (marked in blue, generally above) and groomed specimens (marked in red, generally below).

quantification (loQ) were calculated from blank samples as recommended in the literature [43]:

• LoD = Xblank + 3*SDblank (1)

• LoQ = Xblank + 10*SDblank (2)

Paper and solvent blank samples were extracted and analysed every 6 injections. No contaminations were measured in the blank samples. Control charts were used to ensure the quality of the results [44].

From the detected compounds, 17 lipids were selected for further studies based on their relative abundance and presence in all studied fingermarks (Table 2). The selected lipids were tentatively identified using their retention time and mass spectra in comparison with previous studies [5] and the NIST08 Database (Gaithersburg, MS).

2.4. Data treatment

The peak area (PA) of each selected lipid (l) has been integrated and normalised by the peak area of the internal standard (IS):

$$\mathrm{NPA(l)} = \frac{PA(l)}{PA(IS)} \quad (3)$$

The obtained values have been used to semi-quantitively compare the obtained results between natural and groomed marks. Excel from Microsoft, Origin from originlab® and Python™ have been used for statistical treatment and visualisation.

Hierarchical cluster analysis (HCA) has been used to classify donors according to the composition of their fingermarks [5]. The distance between the obtained normalized peak areas for the 17 target compounds was measured using Euclidian distance. The complete linkage was done by hierarchical agglomerative clustering using centroid linkage for hierarchical representation. The visualisation of the distance for each cluster is represented in dendrograms.

A correlation heatmap was used with a chromatic scale to show a two-dimensional Pearson correlation (r) matrix between two lipids x and y is given by:

$$r_{xy} = \frac{\sum_{i=1}^{n}(x_i - \bar{x})(y_i - \bar{y})}{\sqrt{\sum_{i=1}^{n}(x_i - \bar{x})^2}\sqrt{\sum_{i=1}^{n}(y_i - \bar{y})^2}} \quad (4)$$

The obtained r values range from 1 (correlated) to −1 (anticorrelated). The mean Pearson value is indicated in the matrix, and a colour scale is used to quickly visualise correlations.

3. Results

3.1. Lipid relative quantities in natural and groomed fingermarks

From the 17 selected lipids, five from the different main types were quantified for comparison purpose with previous studies (see Table 2): two sterols (squalene and cholesterol), a fatty acid (palmitic acid) and two wax esters (myristyl myristate, myristyl palmitoleate). Average quantities were calculated in nanogram per fingermark (ng/FM) for these five lipids in all fingermarks (Table 3). An increase of ca. 60% was observed in the summed quantities of these five lipids between natural and groomed fingermarks (from 1481 to 3979 ng/FM, respectively). Squalene was 1–40 times (in average a little more than 4 times) higher in groomed compared to natural marks. A similar increase was also observed in a previous study [23], with the amount of squalene being 1.5–68.4 times larger in groomed compared to natural fingermarks. Another study [20] estimated a 6–12 times increase in

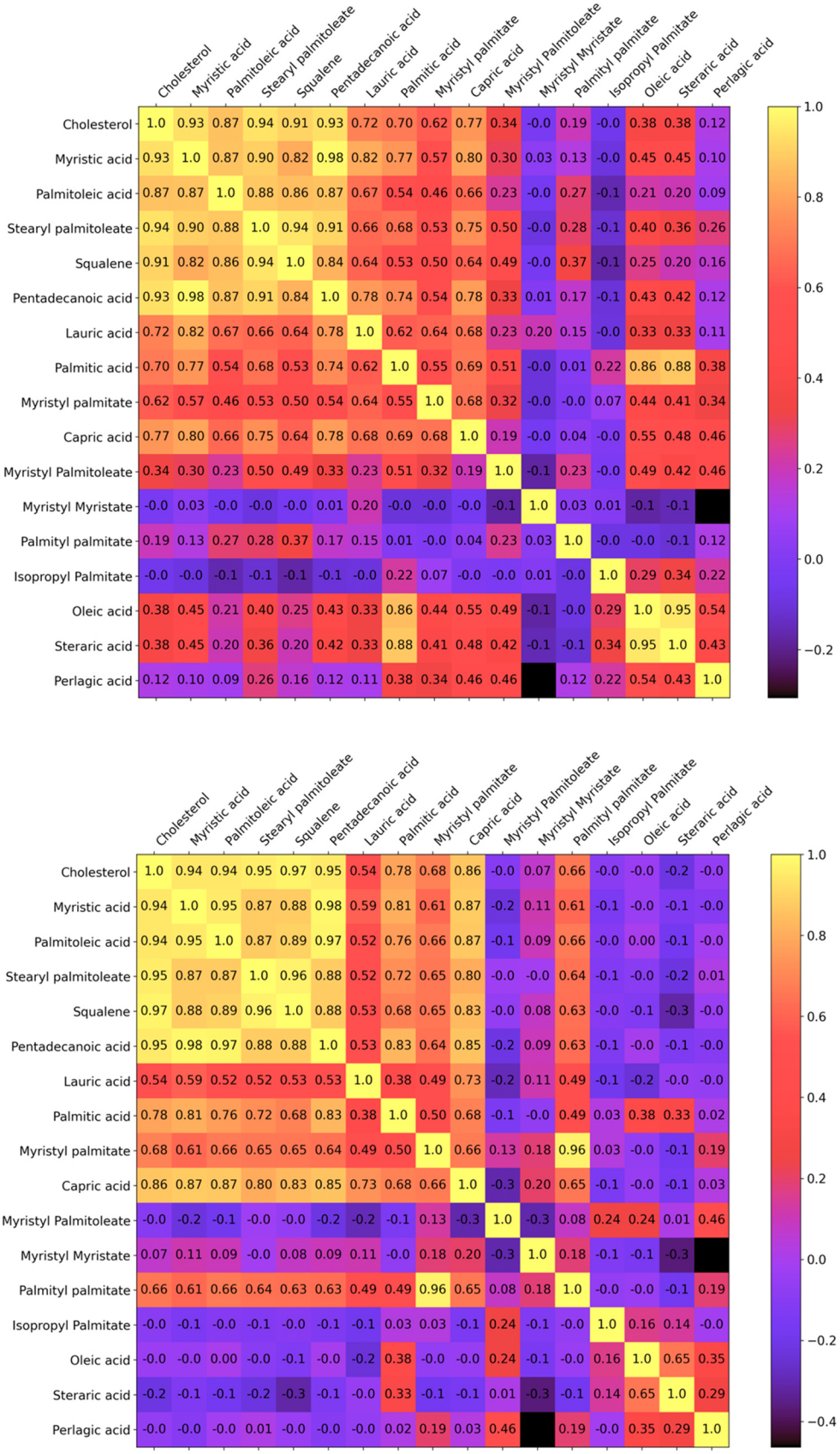

Fig. 4. - Pearson correlation matrix of 17 target lipids in natural (above, n = 50) and groomed (below, n = 51) fingermarks.

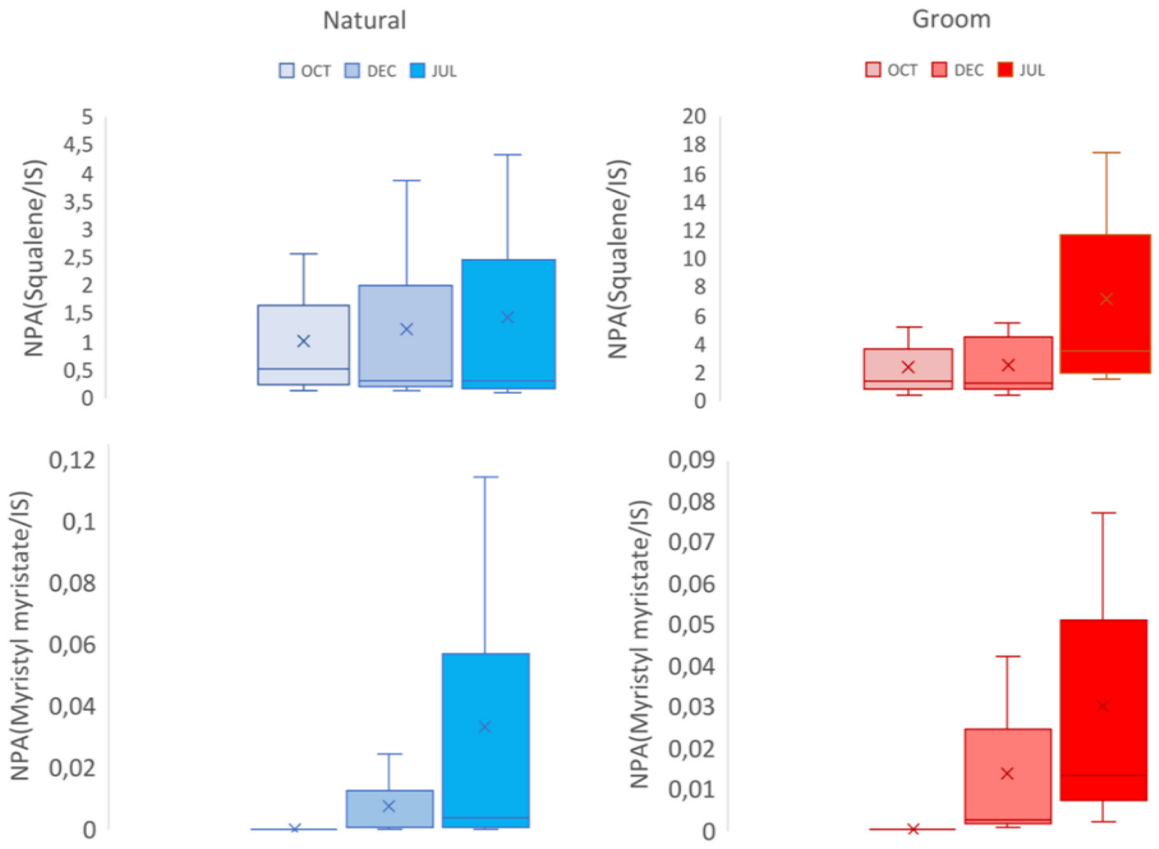

Fig. 5. – Boxplots of the NPA values obtained for squalene and myristyl myristate. Both lipids had higher average (and to some extent median) in July particularly in groomed (right) compared to natural fingermarks (left).

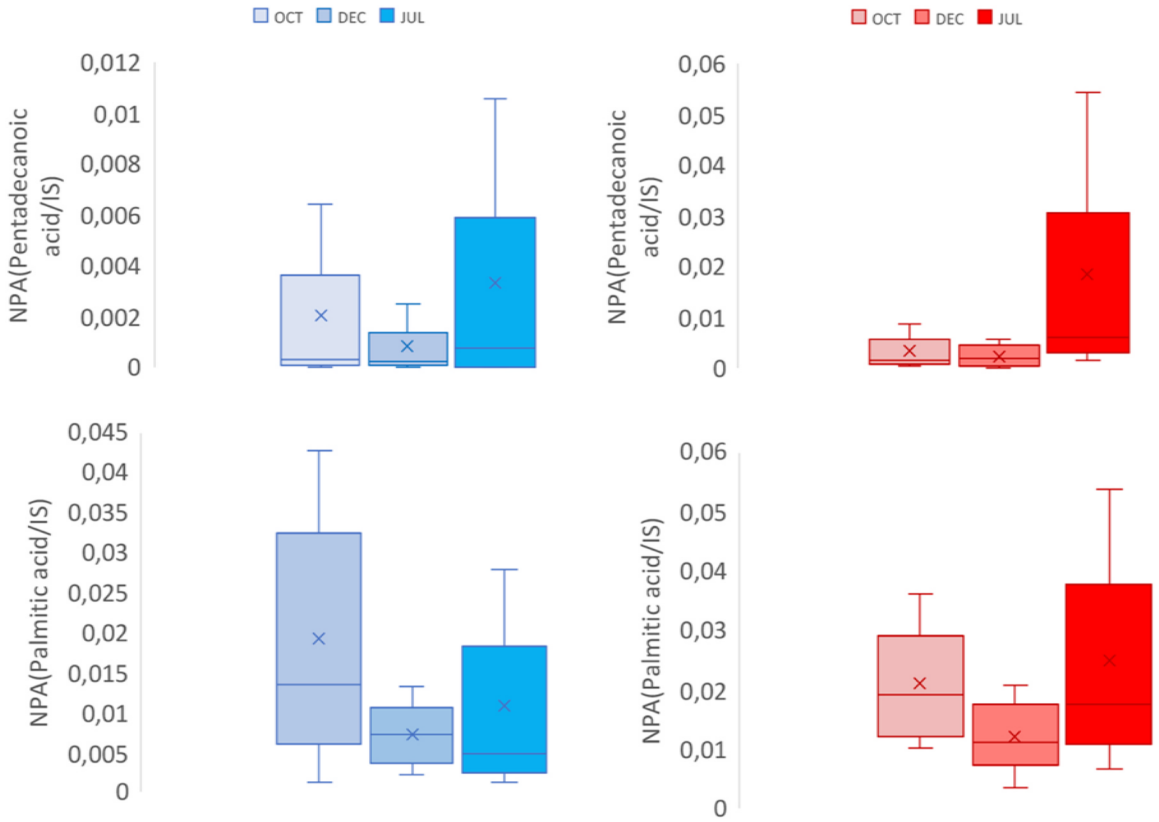

Fig. 6. – Boxplots of the NPA values obtained for pentadecanoic acid and palmitic acid. Both lipids had higher median (and to some extent average) NPA values in October particularly in natural fingermarks (left).

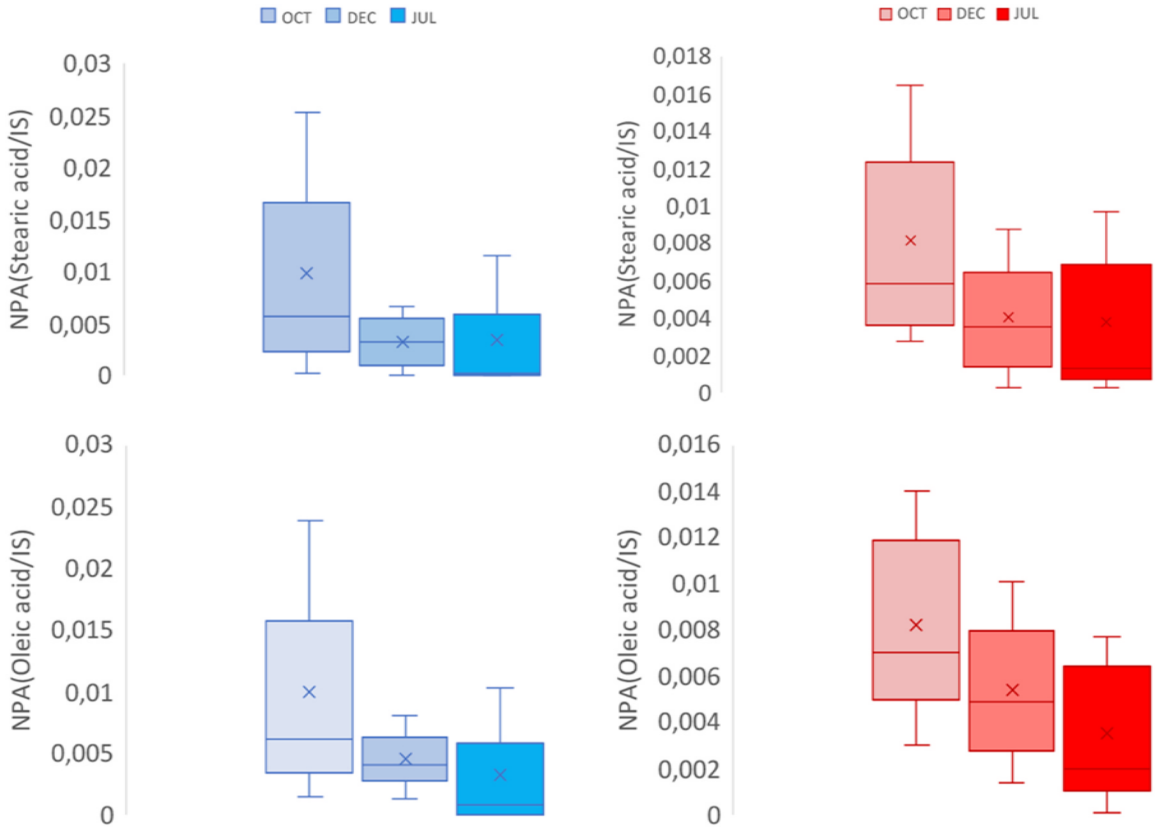

Fig. 7. – Boxplots of the NPA values obtained for stearic and oleic acids, showing decreasing median (and to some extent average) quantities from October to July both in natural (left) and groomed (right) marks.

putative LC/MS-MS fragments from squalene, cholesterol and fatty acid methyl esters.

On average, squalene was the most abundant lipid both in natural and groomed fingermarks, followed by palmitic acid and cholesterol. Similar results have been observed for groomed residue in previous studies [5,9,20,23,25,29,66]. These three compounds are found in the sebum through body synthesis and food consumption [27,28,32,46,47,52,60,67]. However, they can also be found to some extent in cosmetics (i.e., skin products mimicking skin composition) [9,47]. While squalene represents less than 40% of the five lipids in natural fingermarks, it increased to almost 60% in groomed marks (Table 3), indicating that groomed fingermarks were proportionally more saturated in squalene than natural marks. This may either be explained by a higher transfer rate of squalene when deliberately touching the forehead and/or a slower loss after the transfer [5,68]. Myristyl myristate and myristyl palmitoleate were proportionally less abundant in both natural and groomed marks (2–5%).

The relative standard deviations (RSD) of squalene, palmitic acid and cholesterol were higher than those of the wax esters (see Table 3). Lipid quantities showed considerable variability between fingermarks (see Fig. 1). The fingermarks from one of the donors always contained much more of these lipids than the other five donors, explaining the large spread of the data (i.e., the upper whiskers in the boxplots).

The normalised peak areas (NPA) of the 17 targeted lipids are reported in Table 4. The lipids with the larger NPA in natural fingermarks (e.g., squalene, palmitoleic acid and palmitic acid) also had large NPA in natural marks. And inversely, the lipids with the lowest NPA in natural marks (e.g., myristyl palmitoleate, pentadecanoic acid, capric and lauric acid) generally had low NPA in groomed marks. Squalene led to the highest NPA in all 101 analysed fingermarks.

The proportion of all lipids was higher in groomed compared to natural fingermarks (see Fig. 2). The highest difference was obtained for palmitoleic acid (86% in groomed fingermarks) and the lowest for stearic acid (54% in groomed fingermarks). These results confirmed that grooming the fingertips with sebum from the face increased the relative quantities of lipids, but not in the same proportions for all lipids. The averaged NPA of all lipids was four times higher in groomed marks compared to natural fingermarks (see Table 4). As expected, results obtained for natural fingermarks lead to higher RSD than for groomed marks except for myristic acid (see Table 4 and Fig. 3). RSD values between 46% and 266% were obtained (the minimal value was obtained for pelargic acid in groomed fingermarks and the maximum for myristyl myristate in natural fingermarks).

3.1.1. *Lipid correlation in natural and groomed fingermarks*

Correlation between the normalised peak areas of the lipids was studied using Pearson correlation coefficient (r, see Fig. 4). Six compounds, cholesterol, myristic acid, palmitoleic acid, stearyl palmitoleate, squalene and pentadecanoic acid, were strongly correlated both in natural ($r > 0,82$) and groomed ($r > 0,87$) fingermarks.

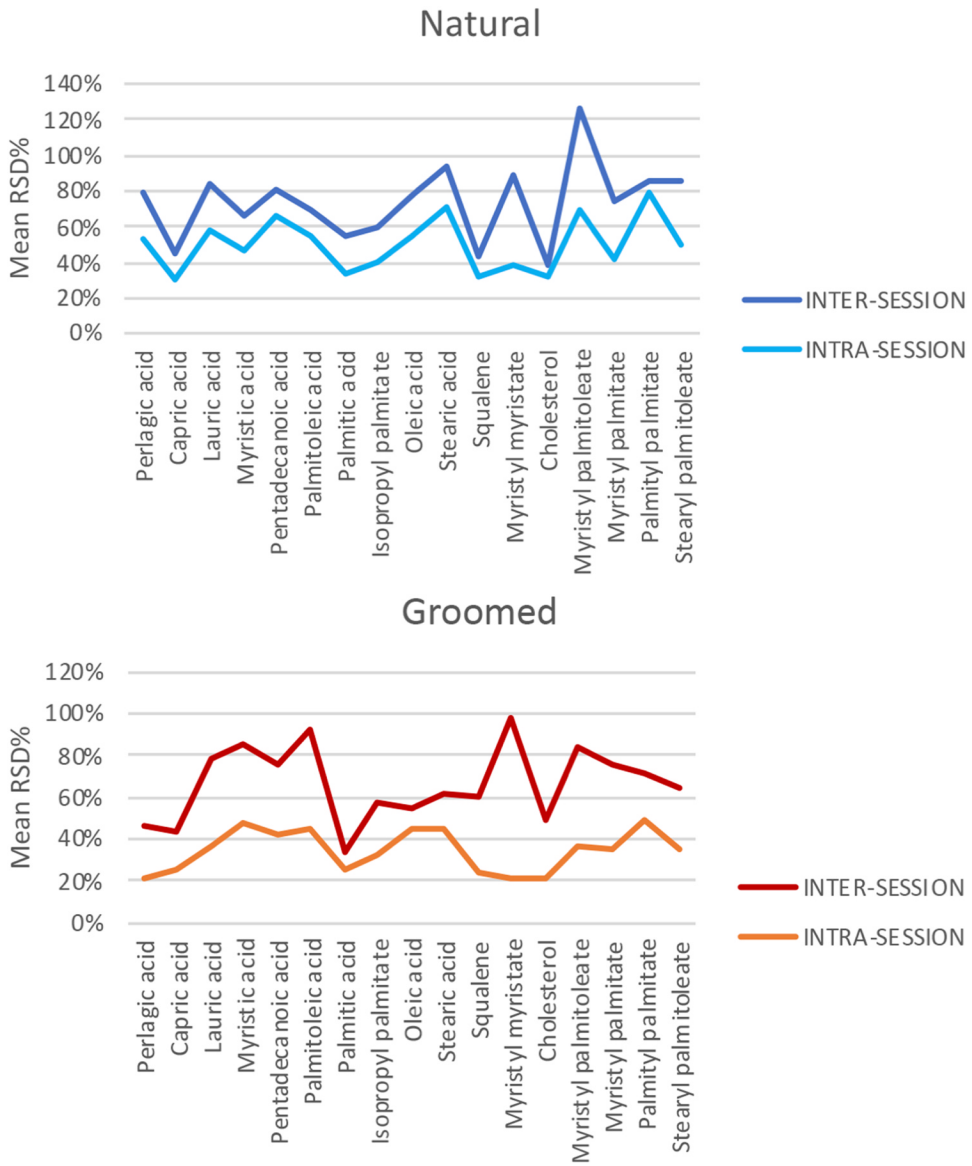

Fig. 8. - Comparison of the mean relative standard deviation (RSD) of the 17 target compounds for natural (above) and groomed (below) fingermarks. Mean RSD values were obtained for the three fingermarks of each donor collected within a session (intra-session) and between sessions (inter-session).

As it can be seen in Fig. 4, four additional compounds (lauric acid, palmitic acid, myristyl palmitate and capric acid) also showed some correlation with the six previously mentioned lipids ($0{,}38 < r < 0{,}87$). Stearic acid and oleic acid were also correlated together ($r = 0{,}95$ in natural and $r = 0{,}65$ in groomed fingermarks). These two compounds also showed some correlations with several of the previously cited lipids in natural marks. Palmityl palmitate was also correlated with some lipids but mainly in groomed marks (r values up to 0,96). The remaining lipids (i.e., myristyl palmitoleate, myristyl myristate, isopropyl palmitate, and pelargic acid) showed little to no correlation with other lipids. Interestingly, most of the correlated lipids are those present in significantly higher proportions in groomed marks (see Fig. 3). Some of the correlated lipids are also known to be correlated in human physiology. For example, saturated fatty acids tend to increase the cholesterol concentration in plasma, and squalene is one of the cholesterol precursors [69,70]. Pentadecanoic acid is found in butter made from cow milk and is also known to increase cholesterol levels [51]. Stearic acid is dehydrogenated to the monounsaturated derivative oleic acid, explaining their positive correlation [70–73].

3.1.2. Session influence

Fingermarks were collected during three sessions at different times of the year (see boxplots for all compounds in the supporting information SI-1). The summed NPA of all lipids increased in the Northern-hemisphere summer session (July) compared to October and December results, indicating that the warmer weather led to higher lipid content in both natural and groomed fingermarks. However, this difference was mainly due to squalene and was more pronounced in groomed than natural fingermarks (see Fig. 5).

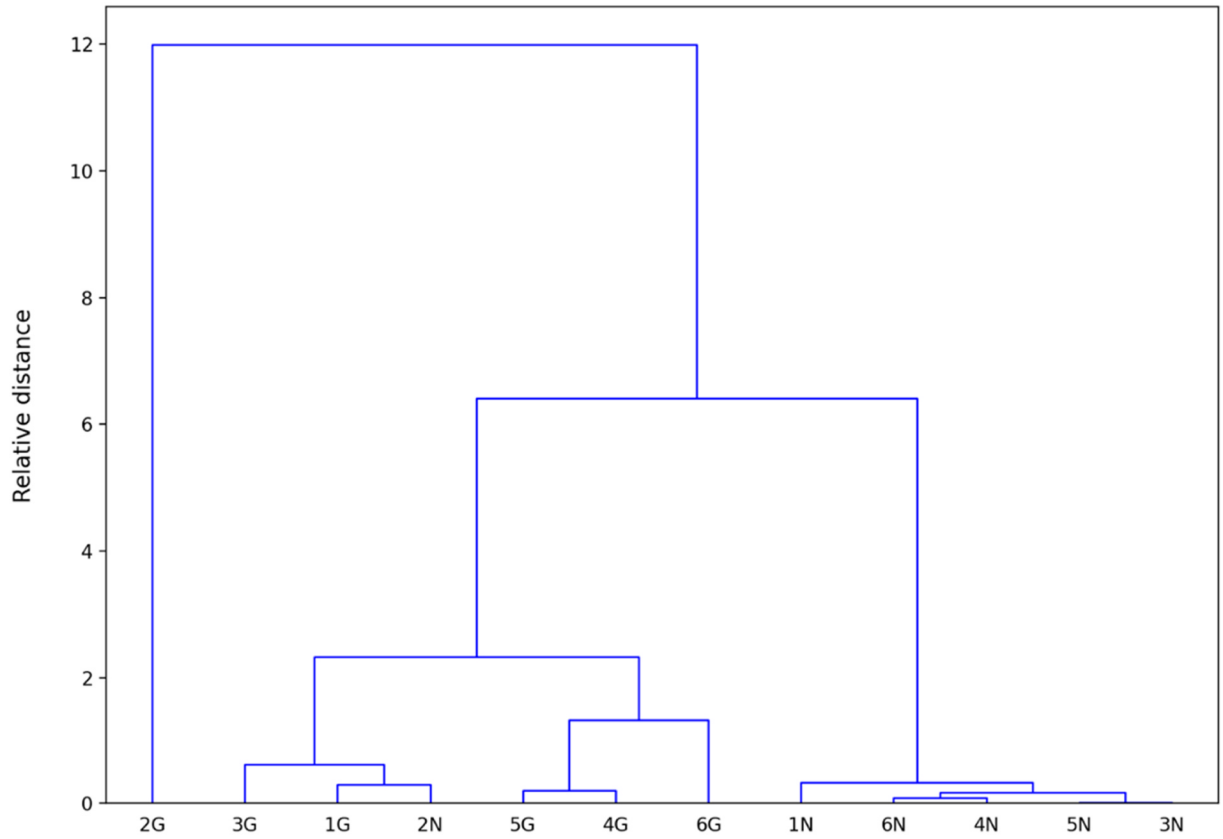

Fig. 9. – Hierarchical cluster analysis with Euclidean distance and complete linkage clustering for average normalised peak areas (NPA) of all lipids per donor. The donors were classified from higher (left) to lower average NPA values (right). Donors were attributed numbers (from 1 to 6), G= groomed, N = natural fingermarks respectively.

Several compounds (especially those correlated to squalene) also showed higher average NPA in July (see Fig. 5 and SI-1): cholesterol, myristic acid, palmitoleic acid, stearyl acid, lauric acid, myristyl palmitate, capric acid and myristyl myristate. While one particularly good donor often influenced the average NPA of these compounds, the median was not consistently higher in July, especially for natural fingermarks (see squalene, for example). Among the correlated compounds, pentadecanoic acid and palmitic acid did not show the same seasonal trends, as their median NPA were higher in October rather than in July (see Fig. 6). The following compounds showed the lowest average (and generally also median) NPA in July (see SI-1): myristyl palmitoleate, isopropyl palmitate, stearic, oleic and pelargic acids. Finally, palmityl palmitate did not show any particular trend between seasons (see SI-1). (Fig. 7).

The mean RSD calculated from the results obtained for each donor within a session (intra-session) were lower than those obtained between sessions (inter-session) confirming that the composition of fingermarks collected over several sessions was very variable even for a same donor (Fig. 8).

Correlation matrixes were also generated per season (see SI-2). The six highly correlated lipids (cholesterol, myristic acid, palmitoleic acid, stearyl palmitoleate, squalene and pentadecanoic acid) were correlated for all seasons and types of residue ($0{,}53 < r < 0{,}99$). The average correlation of those six lipids was slightly higher in July compared to October and December both in natural and groomed fingermarks. The average correlation values were generally close for natural and groomed marks, except in December (a few correlations values were relatively lower, for example for pentadecanoic acid).

Stearic and oleic acid were generally correlated, but always less in groomed marks compared to natural ones, and more in October comparatively to the other months: $r = 0{,}97/0{,}88$ in October, $r = 0{,}75/0{,}59$ in December, and $0{,}88/-0{,}2$ in July (for natural/groomed fingermarks respectively). Palmitic acid was also generally correlated to stearic and oleic acids, but less in groomed marks than natural ones [74]. These results indicate that these fatty acids may be less correlated when the fingermarks were groomed and during warmer weather. All other compounds were more or less correlated depending on the collection sessions, without particular trends (see SI-2). For example, the following compounds showed less correlation for some collection sessions: lauric acid in July, palmitic acid in October and December, and capric acid in December.

3.1.3. Donor classification

A donor classification model based on the lipid composition of groomed fingermarks provided by 25 donors was previously proposed [5]. As groomed fingermarks contained proportionally more lipids than natural ones, the robustness of the previously proposed classification model has been tested for natural fingermarks, as well as between different deposition sessions.

In general, groomed fingermarks led to higher average NPA compared to natural ones, except for donor 2, for which the average NPA of natural fingermarks was classified higher than most groomed

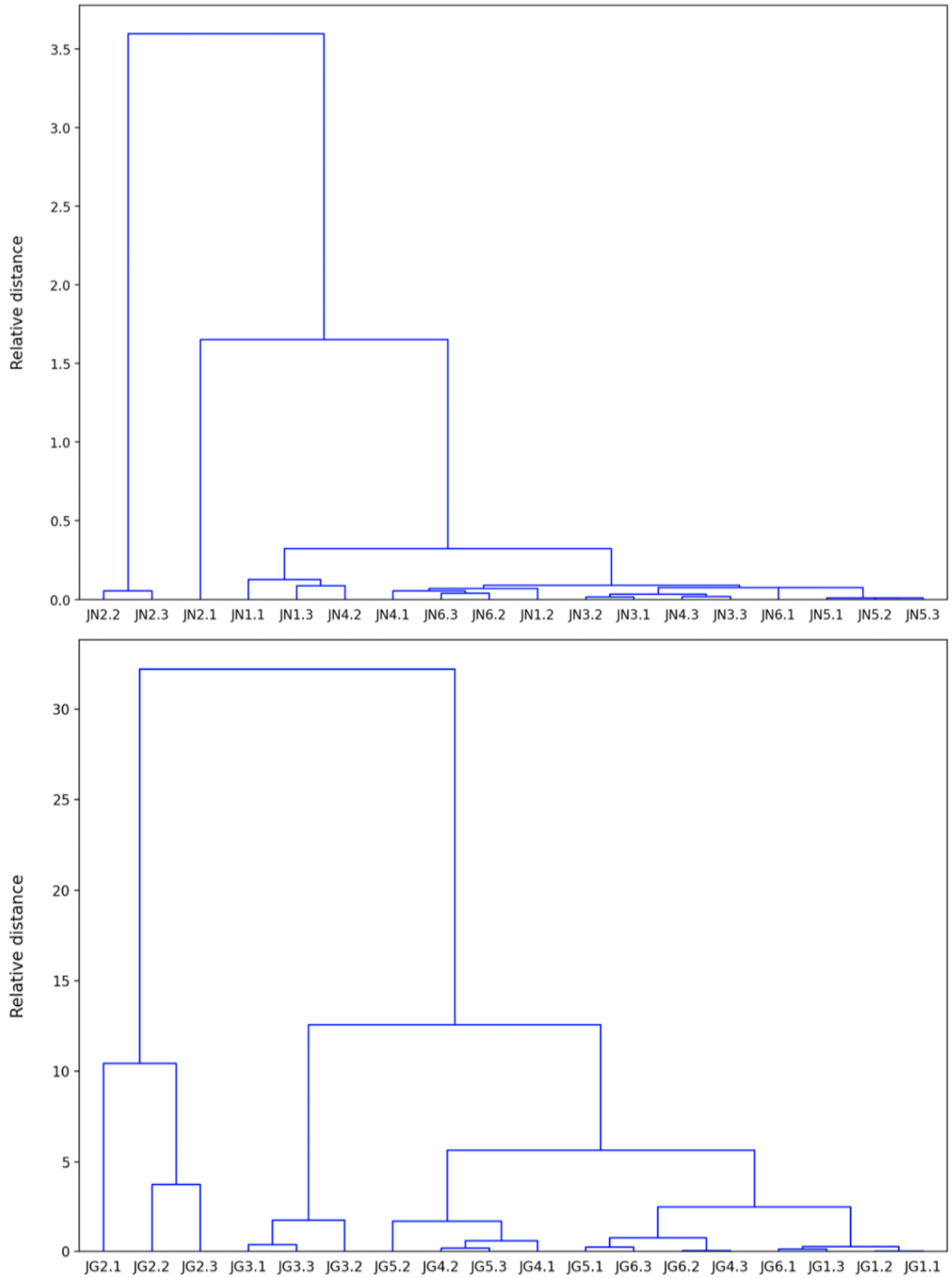

Fig. 10. – Hierarchical cluster analysis with Euclidean distance and complete linkage clustering for average normalised peak areas (NPA) of all lipids per donor (#1–6 with triplicate fingermarks indicated from.1 to.3) for the July (J) deposition session. The specimens were classified from higher (left) to lower average NPA values (right). Hierarchical clusters were obtained for natural (N, above) and groomed (G, below) fingermarks respectively. Triplicate finermarks for a same donor were not always grouped together (see for example donor 1 above and donor 4 below).

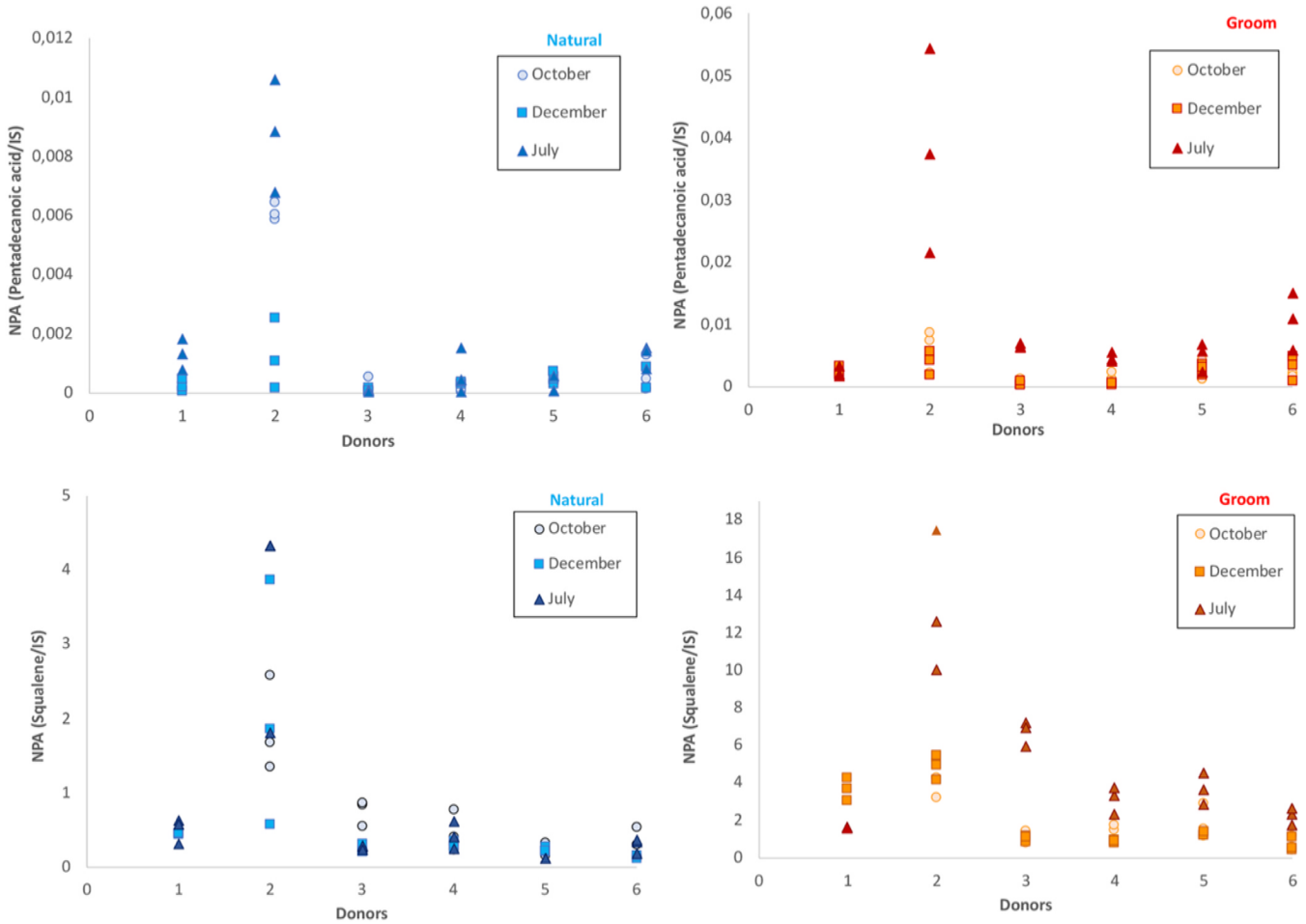

Fig. 11. – Normalised Peak Area (NPA) values for pentadecanoic acid and squalene. The 9 values obtained for each donor (2–6) are plotted and marked as a function of the collection session. Donor 1 was missing in the October session, thus only 6 values were plotted for this donor.

marks from other donors (see Fig. 9). The classification was mainly influenced by the squalene content. Thus, a donor with a relatively high squalene content will be classified as a “good” donor, while a donor with a lower amount of squalene will be classified as a “poor” donor.

In this study, donor 2 was the "best" donor in all conditions for both natural and groomed, fingermarks (see Fig. 9, SI-3 and Fig. 10). Donor 1 was not present during the October session, but was the “second-best” donor in December, as well as for the natural fingermarks collected in July together with donor 4. However, donor 1 was the “poorest” donor for the groomed marks collected in July. The classification of the other donors differed significantly between natural and groomed residues and deposition sessions (see Fig. 10 and SI-3). For example, while donor 5 was a "relatively good" natural fingermark donor in December compared to other donors (SI-3), he/she was the "poorest" natural fingermark donor in July (see Fig. 10) and pretty "average" in most of the other situations (i.e., neither the “best” nor “the poorest” donor, thus showing no real "good" or "poor" donor trend). It seems that, except for very "good" lipid donors (e.g., donor 2 in this study), the previously proposed classification model only gives a very approximative indication of the average lipid content (mainly squalene) of a trace compared to others. In fact, replicate specimens from the same donor were not always clustered together even if the lipid content was homogenised and collected at the same time, indicating a variability in the transferred residue that remained very difficult to control even for groomed marks (see for example the relative classification of natural specimens of the donors 1, 3, 4 and 6 and the groomed specimens of the donors 4, 5 and 6 collected in July in Fig. 10).

When observing the replicate results obtained per donors (Fig. 11), donor 2 was a particularly good squalene donor particularly in July. Donor 2 was also the best donor for the correlated compounds (cholesterol, myristic acid, palmitoleic acid, stearyl palmitoleate and pentadecanoic acid) and for most of the other compounds mainly in July (see Fig. 11 and SI-4).

However, the fingermarks of other donors contained higher proportions of some compounds for some seasons (see Fig. 12 and SI-4). For example, donors 1 and 5 were the best myristyl myristate donors in July and October, respectively. Some compounds were found in comparable quantities in the different marks such as pelargic acid, myristyl palmitate, stearic and oleic acids.

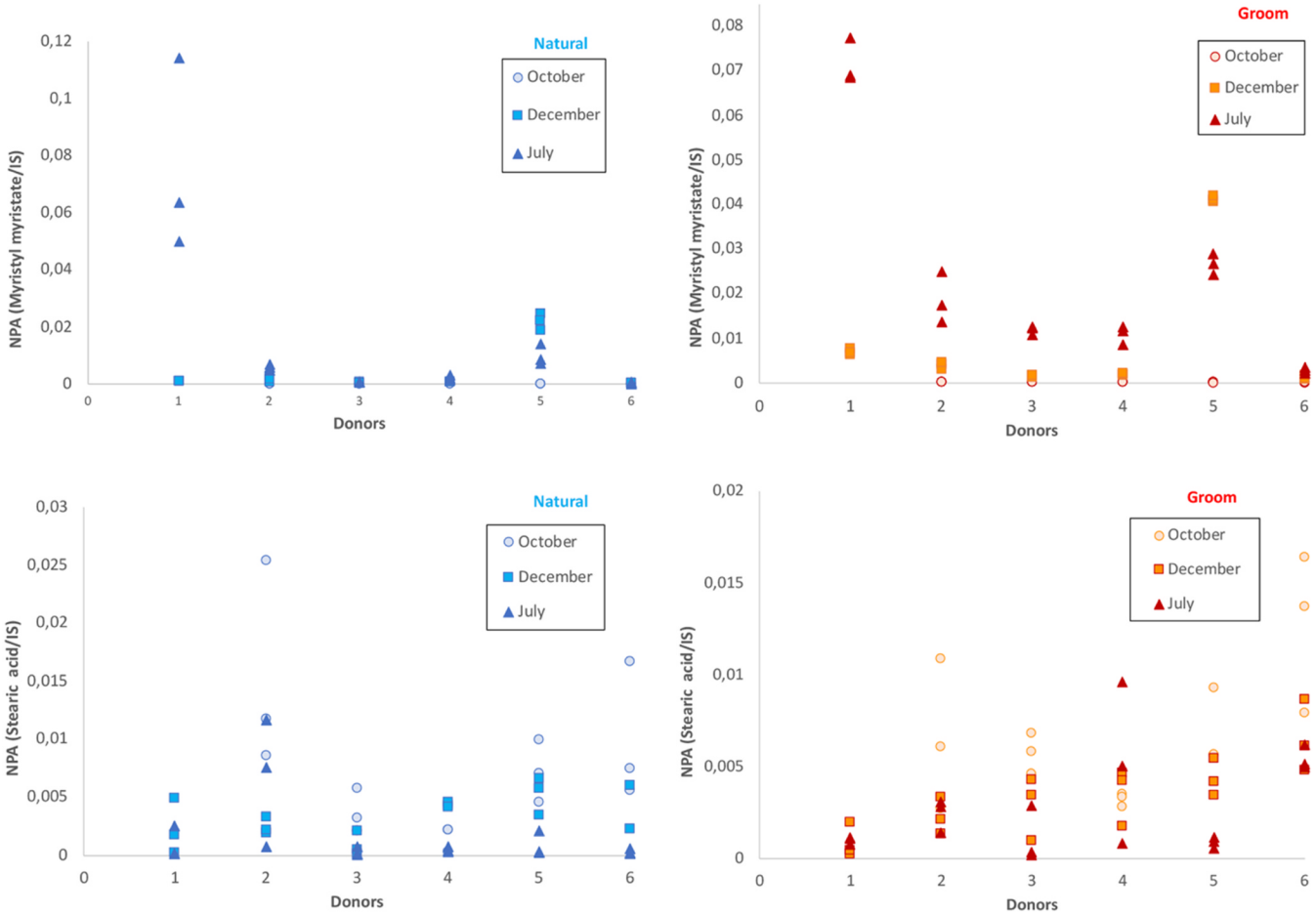

Fig. 12. – Normalised Peak Area (NPA) values for myristyl myristate and stearic acid. The values obtained for each donor (1–6) are plotted and marked as a function of the collection session.

4. Conclusions and perspectives

This work investigated and compared the lipid composition of natural and groomed fingermarks. A total of 101 (50 natural and 51 groomed) fingermarks were collected from 6 donors during 3 sessions and analysed using GC/MS.

As expected [23] natural fingermarks generally contained less lipids than groomed fingermarks. Squalene was the major compounds in all marks, particularly in groomed specimens. On average, groomed fingermarks contained four times the proportion of lipids found in natural ones. This was less marked for some of the studied lipids (i.e., myristyl myristate, isopropyl palmitate, palmitic, capric, pelargic, oleic and stearic acids) for which the proportion in groomed marks was only slightly higher than in natural ones. The lipid composition of natural fingermarks showed more variation compared to groomed marks for all compounds except myristic acid. Variability was higher for a donor between sessions than within sessions, and the variation between donors over three collection sessions was very high (RSD values above 100% for most compounds).

The influence of the ambient temperature (warmer in July than in October and December) can influence the sebum secretion (i.e., perspiration). It was observed that some compounds, such as squalene and cholesterol, were indeed more abundant in July particularly in marks groomed with sebum. Other compounds, such as stearic and oleic acids were more abundant in October.

Some of the lipids found in higher proportions in the groomed fingermarks were correlated together (i.e., cholesterol, myristic acid, palmitoleic acid, stearyl palmitoleate, squalene and pentadecanoic acid), while those with similar proportion in natural and groomed marks were also often correlated together (i.e., oleic, stearic and to some extent palmitic acid). This observation potentially points at different main sources, mainly endogenous (from the sebum) for the first six first, while the last three may also have significant exogenous sources (e.g., food consumption or cosmetics).

Major differences in the fingermarks lipid content were also observed between donors. The fingermarks of one of the donors generally contained higher relative amounts of lipids both in groomed and natural residue compared to the others. All other fingermarks led to very variable amounts and did not allow to classify the other donors as constantly "good" or "poor" donors.

In summary, lipid content is very variable within and between donors, particularly in natural marks. However, the fact that the lipid content of natural marks should be closer to residue encountered in practice than groomed marks may not be entirely true. Indeed, Locard suggested that the intensity of the criminal act led to the creation and transfer of more traces [75]. Thus, an intense criminal activity could also lead to more sweating, and thus potentially more incident-driven grooming of the fingermarks.

In any case, many lipids are systematically found in both natural and groomed fingermarks (at least a few hours after deposition), squalene being by far the most abundant of those studied in this work. A previous work indicated that squalene was still found in studied fingermarks one month after deposition [76]. Thus, while all the studied lipids may be interesting targets for detection techniques, squalene probably plays a major role in the detection

mechanisms. These results may be particularly useful to better understand detection techniques in which lipids are known to play a role, with regards to the detection mechanism (e.g. of the physical developer) or decreasing efficiencies with time (e.g. ORO) [16]. Further studies, including older fingermarks, will be needed to better understand the role of squalene and other lipids (particularly triglycerides that were not detected with GC/MS) in the functioning of physical developer. These results may also be useful to develop adequate artificial fingermarks secretions to further support the development of those detection techniques [18].

CRediT authorship contribution statement

Ana Moraleda – conceptualization, data curation, data treatment, writing – original draft, Claude Roux – conceptualisation, writing – review & editing, workplan, Andy Bécue – conceptualisation, writing – review & editing, workplan, Céline Weyermann – funding acquisition, conceptualisation, project administration, writing – original draft.

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Conflict of interests

All authors declare that they have no conflicts of interest.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.forsciint.2023.111709.

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