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1 **Changes in latent fingerprint glyceride composition as a function of sample age using UPLC-IMS-**
2 **QToF-MS^E**

3

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8

9 **Abstract**

10 The composition of fingerprint residue has been an important topic in forensic science, mainly in
11 efforts to better understand and eventually improve the efficacy of latent fingerprint detection
12 methods. While the lipid fraction has been extensively studied, there is currently little information
13 about how the glyceride fraction of latent fingerprints is chemically altered over time following
14 deposition. A previously reported untargeted ultra performance liquid chromatography-ion mobility
15 spectrometry-quadrupole time-of-flight mass spectrometry (UPLC-IMS-QToF-MS^E) method was used
16 to investigate changes over time in fingerprint di- and triglycerides. Charged latent fingerprint
17 samples from 5 donors were analysed up to 28 days following deposition. Significant changes in
18 glyceride composition occurred with increased sample age, attributed primarily to the oxidation of
19 unsaturated triglycerides through ozonolysis. Considerably fewer unsaturated TGs were identified in
20 samples 7 and 28 days following deposition, while mono- and diozonides of these lipids were
21 identified as major components of aged samples. Additional compounds were identified as possible
22 aldehyde and dicarboxylic acid derivatives resulting from the reaction of water with ozonolysis
23 intermediates. While the onset of these processes occurred rapidly following deposition, continuing
24 oxidation over time was seen via the progressive ozonolysis of diunsaturated triglycerides. These
25 results represent a further step towards understanding the factors affecting fingerprint composition,
26 ageing and subsequent detection under operational conditions.

27

28 **Introduction**

29 There is a general lack of information concerning the ageing of latent fingerprints, in regards to
30 degradation pathways, the products which are formed, and the contribution of environmental factors
31 [1-4]. Following deposition on a surface, latent fingerprint residue is susceptible to chemical and
32 physical modifications that may have a significant impact on the persistence of target compounds, and
33 hence detectability. Investigations into changes in latent fingerprint composition over time have
34 focused on several broad goals: 1) to understand the impact of ageing processes on fingerprint
35 detection [4-7]; 2) to identify degradation products as potential targets for new detection methods [4,
36 6] 3) to develop a reliable method for estimating the age of a fingerprint [2, 3, 8-13].

37 Latent fingerprint residue contains a number of compounds which are liable to undergo significant
38 chemical and physical changes over time following deposition. The nature and rate of these processes
39 can depend on a number of factors, including the type of surface (porous or non-porous) on which the

40 fingerprint is deposited, the environmental conditions to which the fingerprint is exposed (e.g.
41 temperature, humidity, light), and the initial composition of the residue [5, 10, 14-18]. As a result,
42 depending on the above factors and the amount of time that has elapsed since deposition, fingerprint
43 detection may become more difficult, as the target compounds are transformed or removed, or the
44 bulk residue no longer possesses physical properties compatible with the detection method (e.g.
45 viscosity and adhesiveness) [6, 14, 19]. Additionally, the fingerprint pattern itself can be affected due
46 to 'drying' of the residue resulting in the loss of ridge continuity [20], or diffusion of certain fingerprint
47 components through or across the substrate [14, 19, 21].

48 The sebaceous lipids largely comprise what is referred to as the 'fragile' or 'labile' fraction of the
49 water-insoluble fingerprint constituents [22, 23]. Unlike the more durable 'robust' fraction (large,
50 insoluble proteins and lipoproteins), the labile fraction is readily oxidised and eventually degraded to
51 volatile organic compounds, depending on environmental conditions [3, 22, 24-26]. Several broad
52 trends have been identified regarding the lower molecular weight and unsaturated lipid components.
53 The rapid oxidation of squalene, a highly unsaturated compound, has been observed in many
54 investigations into fingerprint lipid degradation [4-6, 16, 18, 27]. The rate of squalene loss is
55 accelerated by light exposure [5, 27]. Cholesterol has been shown to decrease at a slower rate [16].
56 Further indications as to the kinds of chemical changes undergone by latent fingerprint residue may
57 be found in dermatological research, which is concerned primarily with *in situ* skin surface lipids.
58 Ozonolysis and photo-oxidation of squalene, cholesterol and unsaturated fatty acids have been
59 observed to take place on the skin surface [24, 28-30].

60 For the most part, investigations into latent fingerprint ageing have not utilised approaches amenable
61 to large, neutral lipids, and so it is unclear what chemical changes are undergone by these compounds,
62 and how this may impact upon fingerprint detection. Glycerides (including mono-, di- and
63 triglycerides) comprise approximately 30 % of total skin surface lipids, making them one of the major
64 lipid classes in latent fingerprint residue [31, 32]. The ageing processes of these compounds are
65 relevant to a variety of fingerprint detection techniques, particularly on wetted substrates, where
66 other targets, such as amino acids, may have been removed [33, 34]. While several possible
67 explanations of glyceride degradation exist, few are based on direct observation in fingerprint
68 samples.

69 Archer *et al.* reported that the concentrations of most free fatty acids appeared to undergo an initial
70 increase, followed by a subsequent decrease over a 33 day period [14]. They hypothesised that there
71 might be competing mechanisms of fatty acid generation and degradation. The free fatty acids in
72 human skin surface lipids are derived from triglycerides (TGs) secreted by the sebaceous glands [35-
73 37]. Facultative anaerobes are responsible for the hydrolysis of sebaceous TGs to diglycerides (DGs),
74 monoglycerides (MGs) and free fatty acids within the secretion ducts of the sebaceous glands [38-40].
75 Skin flora from multiple genera may be deposited onto a surface through contact with friction ridge
76 skin [41]. It has therefore been proposed that under certain storage conditions, these bacteria could
77 survive in latent fingerprint residue such that enzymatic processes would continue to affect the initial
78 fingerprint composition [1, 3]. The degradation of unsaturated free fatty acids (namely 16:1 and 18:1)
79 has been reported by other researchers [3, 6], however the trend described by Archer *et al.* was not
80 observed. Conversely, Weyermann *et al.* found no significant changes in free fatty acid concentration
81 [16].

82 Unsaturated sebaceous TGs can contain as many as 9 double bonds, providing multiple target sites for
83 oxidation [42-44]. Mong *et al.* observed that with increased age, latent fingerprint residues appeared
84 to become solidified and less receptive to staining by histological dyes. This was attributed to
85 autooxidation of the unsaturated TGs, via mechanisms postulated to be similar to that of drying oils

86 [6]. Such processes involve the formation of glyceride hydroperoxides, followed by crosslinking to
87 form insoluble polymers [45, 46], but this has never been described in latent fingerprints. Johnson
88 and Rogers similarly inferred that increases in the OH stretch band in fingerprints subjected to
89 elevated temperatures may be due in part to the oxidation of unsaturated TGs [47]. More recently,
90 Pleik *et al.* described the formation of ozonides (1,2,4-trioxolanes) from unsaturated TGs under
91 ambient atmospheric conditions [13]. Storage conditions appeared to affect the reaction rate, with
92 ozonolysis occurring much more slowly when fingerprint samples were protected from light and
93 airflow.

94 The lack of fundamental understanding regarding fingerprint ageing means that it is currently difficult
95 to rationally explain how fingerprint detectability may be affected by chemical and physical
96 modifications of the latent residue. Amongst the lipid fraction, there is very little information
97 regarding degradation products or intermediates in latent fingerprints that can be attributed to
98 glycerides. It should be noted that many studies into sebaceous lipid oxidation have each focused on
99 only one group of oxidation products (such as hydroperoxides) [48], and therefore it is uncertain which
100 mechanism(s) take greatest precedence in relation to fingerprint residue. We recently reported a
101 method for the untargeted separation and characterisation of latent fingerprint glycerides using ultra
102 performance liquid chromatography-ion mobility spectrometry-quadrupole time-of-flight mass
103 spectrometry (UPLC-IMS-QToF-MS^E) [49]. This data-independent acquisition approach enabled over
104 100 intact di- and triglycerides to be identified, with only limited prior knowledge of the glyceride
105 species present in fingerprint residue. This paper describes the further application of this approach to
106 investigate the changes in glyceride composition over time within fingerprint samples from a small
107 donor population. The key aims of this investigation were to determine changes in glyceride profiles
108 with increasing sample age, as well as to identify possible degradation products based on known
109 oxidation mechanisms of these lipids.

110

111 **Materials and methods**

112 **Chemicals**

113 Dichloromethane (GC grade; Sigma-Aldrich, Switzerland), acetonitrile (ULC/MS grade; Biosolve,
114 France), water (LC-MS Ultra grade; Honeywell, Germany), 2-propanol (ULC/MS grade; Biosolve,
115 France), formic acid (ULC/MS grade; Biosolve, France), ammonium formate (LC-MS Ultra grade; Fluka,
116 Switzerland), dimyristin (DG 28:0), dipalmitolein (DG 32:2), dipalmitin (DG 32:0), diolein (DG 36:2) and
117 distearin (DG 36:0) (all >99 %; Nu-Chek Prep, Inc, USA), tricaprilin (TG 24:0), tricaprin (TG 30:0),
118 trilaurin (TG 36:0), trimyristin (TG 42:0) and tripalmitin (48:0) (all 99.9 %; Sigma-Aldrich, USA) were
119 used as received.

120 Stock solutions of glycerides were prepared in dichloromethane. A 1 μ M glyceride mixture in 2:1:1
121 isopropanol/acetonitrile/water was used as a quality control standard. All standard solutions were
122 stored at -24 °C before and after analysis to prevent degradation and solvent evaporation.

123 **Sample collection and storage**

124 Latent fingerprint samples were collected from 5 adult donors (27 – 36 years old) on 25 mm filter
125 paper circles (Grade 1 qualitative filter paper; Whatman, UK). Donors were asked to refrain from
126 handling food or chemicals, or washing hands 30 minutes prior to sampling, but were otherwise free
127 to carry out normal activities and use of skin products. Donors were asked to provide charged
128 fingerprints by briefly rubbing the middle three fingertips of both hands on their forehead and nose,

129 then rubbing the fingertips of each hand together to homogenise the secretions. Each of the 6
130 fingertips were pressed gently to individual filter paper circles for approximately ten seconds. Donors
131 were then asked to repeat the charging procedure and deposit a further six samples, providing a total
132 of 60 samples (12 per donor).

133 9 samples from each donor were stored in open Petri dishes (lined with aluminium foil) on an office
134 desk, allowing exposure to natural light and airflow, for up to 28 days prior to extraction. The
135 remaining 3 samples from each donor were stored in a metal desk drawer (located immediately
136 below the light-exposed samples) in the same manner for 28 days. Sets of clean filter papers were
137 also stored with the samples to provide analytical blanks for both ageing conditions. The office was
138 not climate-controlled, exposing samples to variations in weather conditions. Temperature and
139 humidity dataloggers (Elpro, Switzerland) were placed with samples in each storage location and
140 programmed to record measurements every 30 minutes. The temperature and relative humidity
141 measurements were largely similar for both storage conditions (Table 1) throughout the 28 days.
142 Samples stored on the desk were exposed to elevated temperatures for brief periods during clear
143 mornings, due to direct sun illumination through an adjacent window.

	Desk	Drawer
Average temperature (°C)	24.5	24.4
Minimum temperature (°C)	22.9	22.2
Maximum temperature (°C)	40.8	26.2
Average relative humidity (%)	29.6	29.3
Minimum relative humidity (%)	16.7	16.2
Maximum relative humidity (%)	42.8	43.3

144 **Table 1:** Environmental conditions of sample storage locations

145 **Fingerprint extraction**

146 Of the samples stored on the desk, three randomly chosen samples from each donor were extracted
147 1, 7 and 28 days after deposition. The samples stored in the desk drawer were extracted 28 days after
148 deposition. The filter papers were individually placed in 1.75 mL glass screw-top vials (Thermo Fisher
149 Scientific) that had been cleaned by rinsing with dichloromethane and left to air-dry. Samples were
150 immersed in 750 µL dichloromethane for 2 minutes, with gentle manual agitation to ensure that the
151 filter papers were completely submerged in the solvent. After 2 minutes, the extract was transferred
152 to a second vial. Sample cleanup was performed by adding 750 µL water and vortex mixing before
153 allowing phase separation. The aqueous top layer was discarded, together with a small amount of the
154 organic layer. The remaining extract was stored briefly at -24 °C to freeze any residual water before
155 transferring the organic layer to a third vial. The extract was evaporated under nitrogen gas until
156 approximately 150 µL remained. This residue was transferred to an amber glass vial containing a 250
157 µL glass insert, and further evaporated to dryness. The remaining residue was dissolved in 200 µL of
158 2:1:1 2-propanol/acetonitrile/water with 20mM ammonium formate.

159 **Chemical analysis**

160 Chromatographic separation was performed using a Waters Acquity UPLC I-Class system, coupled to
161 a Waters Vion IMS-QToF mass spectrometer equipped with an electrospray ionisation (ESI) source.
162 The UPLC system was equipped with a binary pump, a 96 well autosampler (maintained at 8 °C), and
163 a temperature-controlled column compartment. Separation was performed using an Acquity UPLC
164 CSH C₁₈ column (2.1 x 100 mm, 1.7 µm), connected to an Acquity UPLC in-line filter to protect the
165 column (both from Waters). The mobile phases were A) 60:40 acetonitrile/water with 10 mM
166 ammonium formate and 0.1 % formic acid and B) 90:10 isopropanol/acetonitrile with 10 mM

167 ammonium formate and 0.1 % formic acid. Gradient parameters are described in Table 2. The flow
 168 rate was maintained at 0.4 mL/min with a column temperature of 55 °C. The injection volume was
 169 1 µL. Injections of the blank filter paper extracts were run before and after sample sets from each
 170 donor to monitor for carryover and contamination.

Time (min)	%A	%B
0.0	60	40
2.0	57	43
2.1	50	50
12.0	46	54
12.1	30	70
18.0	1	99
19.0	1	99
19.1	60	40
21.0	60	40

171 **Table 2:** UPLC mobile phase gradient parameters

172 The ESI source was operated in positive mode at a mass resolving power of >40 000 FWHM, using the
 173 following parameters: the source temperature was set to 120 °C, the desolvation temperature to
 174 600 °C, the cone gas flow to 50 L/hr, the desolvation gas flow to 1000 L/hr, and the capillary voltage
 175 to 2 kV. Data were acquired over the *m/z* range of 50 – 1000 with a scan time of 0.2 seconds. A
 176 200 ng/mL solution of leucine enkephalin (*m/z* 556.2766) was used as the lock mass reference and
 177 infused into the ion source at 5 minute intervals. Data acquisition was performed using high definition
 178 MS^E. The low collision energy was set at 6 eV, and the high collision energy ramp at 30 – 60 eV.
 179 Nitrogen was used as the drift gas in the IMS and as the collision gas. Ion mobility and mass calibration
 180 were performed using a Major Mix IMS/Tof Calibration Kit (Waters).

181 Data processing

182 Data were processed using UNIFI (Waters MS Technologies, Manchester, United Kingdom).
 183 Deconvolution and peak picking were performed with 4D peak detection, with a low energy intensity
 184 threshold of 250 counts and a high energy intensity threshold of 100 counts. The fraction of the
 185 chromatographic peak width applied during isotope cluster creation and high-to low energy
 186 association was 0.5. The fraction of the drift peak width applied during cluster creation and high-to
 187 low energy association were 0.5.

188 Identification of TGs and DGs was carried out as described by Frick and Weyermann, using a
 189 combination of *in silico* fragmentation and filtering candidate peaks based on neutral losses of fatty
 190 acyl groups plus ammonia [49]. Based on the fragmentation of TG ozonides reported by Sun *et al.* and
 191 Pleik *et al.* [13, 50], additional expected neutral losses of ozonised fatty acids ($\text{RCO}_{3n+2}\text{H} + \text{NH}_3$, where
 192 $n \leq$ double bonds) were calculated to enable identification of TG ozonides (Table 3). Expected neutral
 193 losses of peroxidised fatty acids ($\text{RCO}_{2n+2}\text{H} + \text{NH}_3$) were also incorporated into the data processing
 194 method. Candidate *m/z* of the potential oxidised glycerides were compared to calculated values for
 195 $[\text{M}+\text{NH}_4]^+$ of hydroperoxides and ozonides of the unsaturated glycerides identified in previous work
 196 [49]. High energy mass spectra were examined to identify predominant fragment ions corresponding
 197 to fatty acid ozonide neutral losses, as well as fragments corresponding to the neutral loss of a fatty
 198 acid and ammonia plus the cleavage of the ozonised fatty acid at the trioxolane ring [13, 50]. Finally,
 199 all candidate glycerides and glyceride oxidation products were required to have a peak response at
 200 least 2.5 times higher than that of any corresponding peak in a preceding blank filter paper extract.

Fatty acid(s)	CN:DB	Ozonide neutral loss
---------------	-------	----------------------

		Formula	Mass (Da)
$\Delta 6$ -tetradecenoic acid	14:1	C ₁₃ H ₂₅ CO ₅ HNNH ₃	291.20457
$\Delta 6$ -pentadecenoic acid	15:1	C ₁₄ H ₂₇ CO ₅ HNNH ₃	305.22022
$\Delta 6$ -hexadecenoic acid (sapienic acid)	16:1	C ₁₅ H ₂₉ CO ₅ HNNH ₃	319.23587
Heptadecenoic acid ($\Delta 6$ - and $\Delta 8$ -heptadecenoic acid)	17:1	C ₁₆ H ₃₁ CO ₅ HNNH ₃	333.25152
Octadecenoic acid ($\Delta 8$ -octadecenoic, oleic and petroselinic acid)	18:1	C ₁₇ H ₃₃ CO ₅ HNNH ₃	347.26717
Octadecadienoic acid (sebaleic and linoleic acid)	18:2	C ₁₇ H ₃₁ CO ₅ HNNH ₃	345.25152
		C ₁₇ H ₃₁ CO ₈ HNNH ₃	393.23627
$\Delta 10$ -eicosenoic acid	20:1	C ₁₉ H ₃₇ CO ₅ HNNH ₃	375.29847

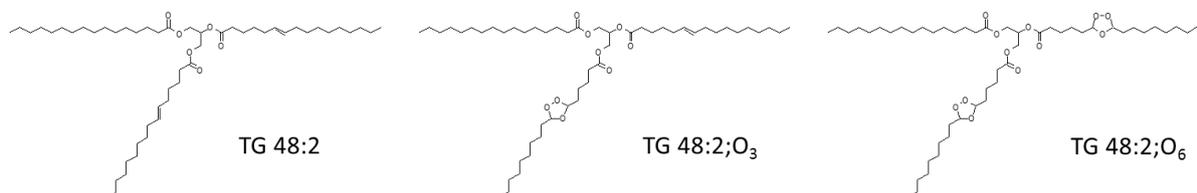
201 **Table 3:** Calculated neutral losses of ozonides of major unsaturated fatty acids identified in human
 202 sebum and latent fingerprints

203

204 Results and discussion

205 Nomenclature

206 Annotation of glyceride structures in this paper follows the recommendations of Liebisch *et al.* [51].
 207 Lipid classes (free fatty acids and di- and triglycerides) are denoted as FA, DG and TG, respectively,
 208 followed by the CN:DB family as a generic identifier (where CN denotes the total number of carbon
 209 atoms in the fatty acyl groups and DB the total number of double bonds). In the case of triglyceride
 210 ozonides, the convention TG CN:DBE;O_x is used (where DBE (double bond equivalent) indicates the
 211 total number of rings and double bonds, and O_x the number of oxygen atoms). As an example, the
 212 monoozonide of the diunsaturated triglyceride TG 48:2 would be expressed as TG 48:2;O₃, and its
 213 diozonide as TG 48:2;O₆ (Figure 1). Where structural information is provided, the separator ‘_’ is used
 214 to indicate that the *sn*-positions of the fatty acids (or equivalent derivatives) are unknown.



215

216 **Figure 1:** Example structural annotations of a triglyceride and its ozonides

217 Changes in glyceride profile with sample age

218 As in previously reported work [49], fingerprint TGs were detected primarily as ammoniated adducts,
 219 eluting over a broad range of 6 – 17 minutes. In total, over 80 families of TGs were identified in 60
 220 fingerprint samples, ranging in structure from 28:0 – 59:2. A summary of the TGs detected in samples
 221 1 – 28 days after deposition is presented in Table 4.

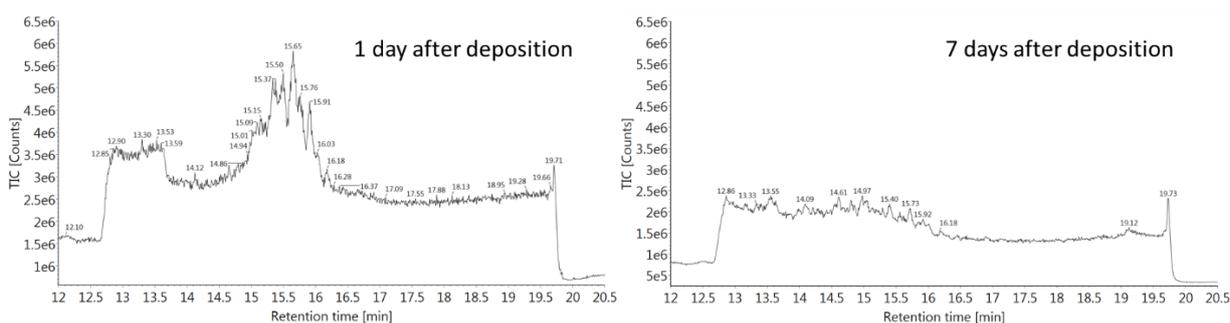
	Sample age			
	1 day	7 days	28 days (desk)	28 days (drawer)
Saturated TGs	26	23	26	25
Monounsaturated TGs	22	10	0	5
Diunsaturated TGs	20	7	4	1

Polyunsaturated TGs	15	0	0	1
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222 **Table 4:** Numbers of triglycerides detected in fingermarks from 5 donors in fingermarks 1 – 28 days
 223 following deposition.

224 The glyceride profiles of 1 day old samples were consistent with previously reported results [49]. The
 225 predominant TGs identified in samples of this age were largely mono and diunsaturated structures,
 226 eluting between 15 – 17 minutes. Saturated and polyunsaturated TGs (containing up to 4 double
 227 bonds) were also present as major glycerides, while DGs were detected as minor components. In four
 228 of the five donors, the glycerides exhibiting the greatest peak signal were unsaturated, ranging from
 229 45:1 – 50:2 in structure. Lower molecular weight glycerides gave greatest peak signals in samples from
 230 the fifth donor, comprising mainly saturated TGs in the range of 32:0 – 40:0.

231 Samples stored for 7 and 28 days after deposition exhibited a dramatic change in glyceride
 232 composition compared to the 1 day old samples (Figure 2). The predominant TGs in these samples
 233 were largely saturated, though some unsaturated TGs were still present at relatively lower levels in
 234 some samples. The rapid decrease of unsaturated TGs is consistent with observations made by Zhou
 235 *et al.* [26]. Similarly, Pleik *et al.* noted the disappearance of TG 48:1 within a few days following
 236 fingermark deposition, though persistence appeared to depend on donor influence [13]. It should be
 237 noted that almost all unsaturated TGs identified in samples stored for 7 – 28 days on the desk
 238 originated from a single donor. This donor reported that they had used moisturising cream and
 239 cosmetics within 12 hours prior to sample collection. While they were not the only donor who had
 240 recently used skin products, samples deposited by this donor contained visible amounts of foundation.
 241 The ingredients of the moisturising cream included sources of glycerides such as shea butter, which
 242 may have contributed to increased triglyceride content in the fingermark samples. The ingredients of
 243 the foundation used by this donor did not include glycerides, but other components of both the
 244 foundation and the moisturiser may have a protective effect on fingermark glycerides, either through
 245 acting as a physical impediment to ageing processes, or by being preferentially degraded. For example,
 246 squalene, which can be present on human skin both as an endogenous component and as a cosmetic
 247 ingredient, is highly susceptible to oxidation processes [4, 24, 26, 52], and may thereby slow the
 248 oxidation rate of the less reactive unsaturated lipids, including TGs [53, 54].



249 **Figure 2:** Total ion chromatograms of UPLC separation of latent fingermark residue from a single
 250 donor, showing differences with increasing sample age.
 251

252 DG species continued to be detected as minor components of some samples throughout the 28 days,
 253 under both storage conditions. As TGs remained the predominant glyceride class in all samples
 254 regardless of age, it can be inferred that the hydrolysis of TGs to DGs and MGs is not a major
 255 degradation pathway of fingermark lipids. In a preliminary study, where samples were stored for up
 256 to 28 days wrapped in aluminium foil, no discernible changes in glyceride content were observed (data
 257 not shown). Glyceride profiles of the wrapped samples were much the same 28 days after deposition

258 as after 1 day, indicating that exposure to air may be a more significant factor in glyceride degradation
 259 than exposure to light. Light exposure has been linked to increased degradation rates of fingermark
 260 compounds such as squalene [5, 27]. However, Pleik *et al.* inferred that the accelerated ozonolysis of
 261 monounsaturated TGs in samples exposed to light might instead be due to competing degradation
 262 mechanisms and additional environmental factors such as increased exposure to air flow [13]. Similar
 263 observations were made by Hinners *et al.* who noted that the rate of TG ozonolysis was significantly
 264 reduced when fingermarks were stored in both clear and opaque containers, compared to samples
 265 left exposed to ambient air [55].

266 Oxidation products

267 Due to the large volume of data obtained during data-independent acquisition, filtering of the data
 268 for candidate degradation products was limited to two mechanisms of TG oxidation: peroxidation and
 269 ozonolysis, as these pathways have been either identified as, or have been hypothesised to be,
 270 possible major pathways of fingermark glyceride degradation.

271 No compounds were identified that corresponded to ammoniated adducts of DG or TG
 272 hydroperoxides, or demonstrated neutral losses of peroxidised fatty acids. While Mong *et al.*
 273 proposed that fingermark glyceride oxidation occurs via similar processes to linseed oil, hydroperoxide
 274 formation is favoured in polyunsaturated fatty acids [45, 46]. Linseed oil, for example, is preferred as
 275 a drying oil due to its faster drying rate, which can be attributed to its high content of linolenic acid
 276 (FA 18:3) [45]. In human sebum, polyunsaturated fatty acids (primarily sebaleic acid and linoleic acid)
 277 are present in trace amounts in comparison to monounsaturated and saturated fatty acids [31, 47].
 278 Monounsaturated fatty acids do not readily undergo autooxidation [56-58], but are susceptible to
 279 rapid reactions with ozone [53]. The peroxidation rate of TGs has been observed to be much lower
 280 compared to other skin surface lipids (notably squalene and cholesterol) [30]. This would explain at
 281 least in part why ozonolysis appears to be a prevalent oxidation pathway of fingermark glycerides and
 282 their free fatty acids [13]. Zhou *et al.* noted that triolein (TG 54:3) did not undergo degradation in the
 283 absence of ozone [53].

284 A number of peaks with m/z consistent with calculated $[M+NH_4]^+$ ions of TG ozonides (i.e. 48 Da or 96
 285 Da greater than a known unsaturated TG) were identified in fingermark samples at all ageing times.
 286 These compounds eluted slightly earlier than the corresponding TGs, over a range of 13 – 16 minutes.
 287 This behaviour in a reversed-phase chromatography separation is consistent with the greater polarity
 288 of the oxidation products compared to their precursor lipids [59]. Notably, a peak was observed at
 289 m/z 870.7391 that exhibited a neutral loss of FA 16:1;O₃+NH₃, which is consistent with the
 290 monoozonide of TG 48:1 (TG 48:1;O₃) described by Pleik *et al.* [13]. By identifying peaks which
 291 exhibited the neutral loss of an ozonised fatty acid plus ammonia, several other candidate ozonides
 292 were identified. In total, 28 peaks were identified corresponding to mono- and diozonides derived
 293 from 23 TGs ranging from 40:1 – 57:1 in structure (Table 5). 17 of these precursor TGs were
 294 monounsaturated, while the remaining 6 were diunsaturated. Of these 23 TGs, 13 had been identified
 295 in previous work as common and abundant within a small donor population [49], from which the 5
 296 donors involved in this study were sourced.

	Average retention time (min)	Average experimental m/z ($[M+NH_4]^+$)	Average CCS (\AA^2) ($[M+NH_4]^+$)	Observed ozonised fatty acid neutral losses	Proposed structure(s)
TG 40:1;O ₃	13.11	758.6140	302.7	FA 14:1;O ₃	-

				FA 16:1;O ₃	
TG 42:1;O ₃	13.68	786.6455	310.2	FA 14:1;O ₃ FA 15:1;O ₃ FA 16:1;O ₃ FA 18:1;O ₃	TG 12:0_14:0_16:1;O ₃
TG 43:1;O ₃	13.95	800.6610	313.8	FA 14:1;O ₃ FA 16:1;O ₃	-
TG 44:1;O ₃	14.15	814.6769	317.6	FA 14:1;O ₃ FA 15:1;O ₃ FA 16:1;O ₃ FA 17:1;O ₃	TG 14:0_14:0_16:1;O ₃ TG 12:0_15:0_17:1;O ₃
TG 45:1;O ₃	14.39	828.6922	321.5	FA 14:1;O ₃ FA 15:1;O ₃ FA 16:1;O ₃ FA 17:1;O ₃	TG 13:0_16:0_16:1;O ₃
TG 46:1;O ₃	14.56	842.7079	324.8	FA 14:1;O ₃ FA 15:1;O ₃ FA 16:1;O ₃ FA 17:1;O ₃ FA 18:1;O ₃	TG 14:0_16:0_16:1; O ₃
TG 47:1;O ₃	14.76	856.7236	328.0	FA 15:1;O ₃ FA 16:1;O ₃ FA 17:1;O ₃	TG 15:0_16:0_16:1;O ₃
TG 48:1;O ₃	14.93	870.7391	332.2	FA 16:1;O ₃ FA 17:1;O ₃ FA 18:1;O ₃	TG 16:0_16:0_16:1;O ₃ TG 14:0_16:0_18:1;O ₃
TG 49:1;O ₃	15.07	884.7549	335.7	FA 15:1;O ₃ FA 16:1;O ₃	TG 16:0_17:0_16:1;O ₃
TG 50:1;O ₃	15.17	898.7705	339.6	FA 18:1;O ₃	TG 16:0_16:0_18:1;O ₃
TG 51:1;O ₃	15.32	912.7861	343.3	FA 15:1;O ₃ FA 16:1;O ₃ FA 17:1;O ₃ FA 18:1;O ₃	TG 16:0_18:0_17:1;O ₃ TG 16:0_17:0_18:1;O ₃
TG 52:1;O ₃	15.47	926.8016	346.6	FA 17:1;O ₃ FA 18:1;O ₃	TG 14:0_20:0_18:1;O ₃ TG 16:0_18:0_18:1;O ₃ TG 17:0_17:0_18:1;O ₃
TG 53:1;O ₃	15.68	940.8184	350.7	FA 16:1;O ₃	-
TG 54:1;O ₃	15.75	954.8332	352.5	FA 18:1;O ₃	TG 16:0_20:0_18:1;O ₃
TG 55:1;O ₃	15.99	968.8493	355.8	FA 16:1;O ₃	-
TG 56:1;O ₃	16.12	982.8653	359.2	FA 16:1;O ₃	-
TG 57:1;O ₃	16.23	996.8803	363.3	FA 16:1;O ₃	-
TG 46:2;O ₃	14.28	840.6930	320.4	FA 14:1;O ₃ FA 16:1;O ₃ FA 18:2;O ₃	-
TG 47:2;O ₃	14.47	854.7094	324.3	FA 16:1;O ₃	-
TG 48:2;O ₃	14.66	868.7238	328.3	FA 16:1;O ₃	-
TG 49:2;O ₃	14.81	882.7391	332.0	FA 16:1;O ₃ FA 18:2;O ₃	-
TG 50:2;O ₃	14.96	896.7543	335.4	FA 16:1;O ₃ FA 18:2;O ₃	-
TG 44:2;O ₆	13.06	860.6461	318.5	FA 18:2;O ₆	-

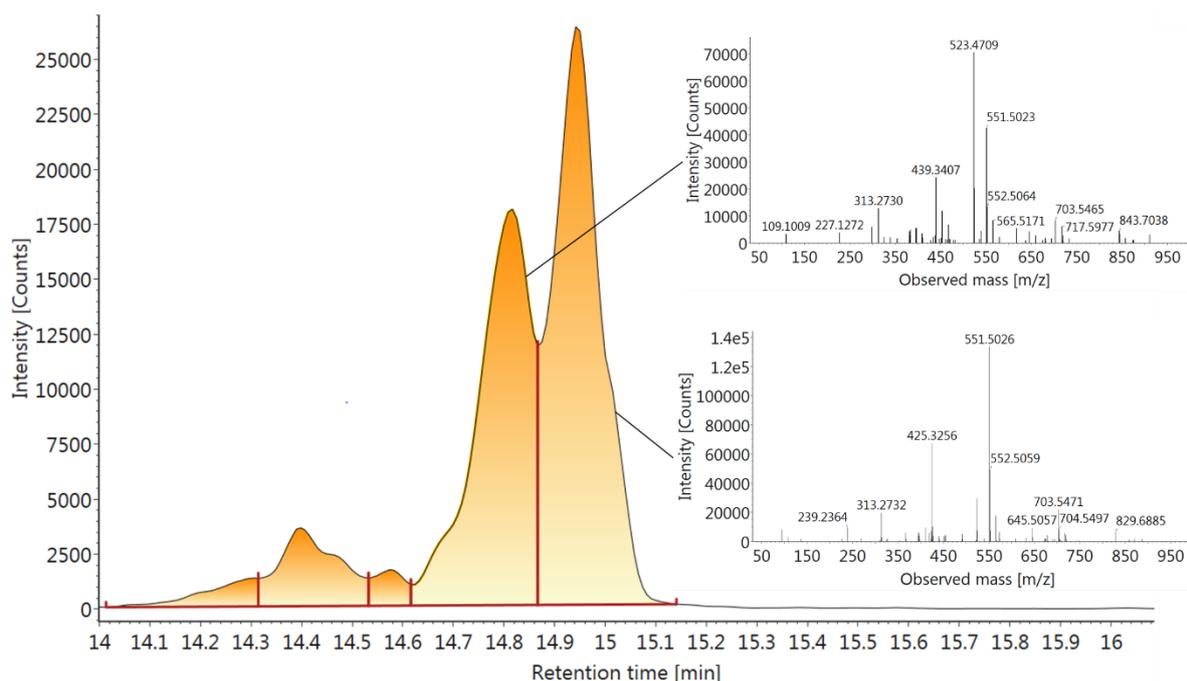
TG 46:2;O ₆	13.53	888.6767	327.1	FA 18:2;O ₆	-
TG 47:2;O ₆	13.78	902.6924	330.9	FA 15:1;O ₃	-
TG 48:2;O ₆	13.96	916.7085	334.5	FA 16:1;O ₃	-
TG 49:2;O ₆	14.05	930.7241	337.5	FA 18:2;O ₆	-
TG 50:2;O ₆	14.24	944.7398	341.5	FA 16:1;O ₃	-

297 **Table 5:** Triglyceride ozonides detected in fingerprints from 5 donors.

298 Collision-induced dissociation of TG ozonides was consistent with previous descriptions [13, 50, 53].
 299 Two major fragment types were observed, corresponding to the neutral loss of the ozonised fatty acid
 300 plus ammonia, and the neutral losses of a fatty acid plus ammonia, together with cleavage of the
 301 ozonide ring. Where one fragment of each type was observed in the high energy mass spectrum of a
 302 TG ozonide, a corresponding structure is proposed in Table 5. Structures were based on the
 303 assumption that the double bond (and therefore trioxolane) position is $\Delta 6$, with the exception of FA
 304 18:1;O₃, where the double bond position was assumed to be $\Delta 8$, as these are the most abundant
 305 positional isomers of each fatty acid in human sebum [3, 31]. However, this list is by no means
 306 exhaustive, as the high energy mass spectra were frequently complicated by multiple diagnostic
 307 fragments of one or both types due to coelution of isomeric TG ozonides, and so structures have not
 308 been suggested for many ozonides. This is not a surprising result, as sebaceous TGs have been shown
 309 to consist of a complex mixture of isomers [49, 60].

310 TG ozonides were frequently affected by peak splitting, which at least in part appeared to be a donor-
 311 dependent issue. Similar observations were made by Sun *et al.*, who attributed this to the incomplete
 312 resolution of positional isomers of ozonides produced from a triolein standard [50]. Furthermore,
 313 different sections of the split TG ozonide peaks exhibited different product ions relating to the losses
 314 of different fatty acids containing the trioxolane ring (Figure 3). Multiple ozonised fatty acid neutral
 315 losses, as well as multiple neutral losses of a fatty acid paired with cleavage of the trioxolane group,
 316 were also observed in the high energy mass spectra of TG ozonides that did not exhibit peak splitting.
 317 When TG ozonides eluted as peak clusters, specific data (e.g. retention time and MS data) is shown
 318 only for the peak section exhibiting the highest signal.

319

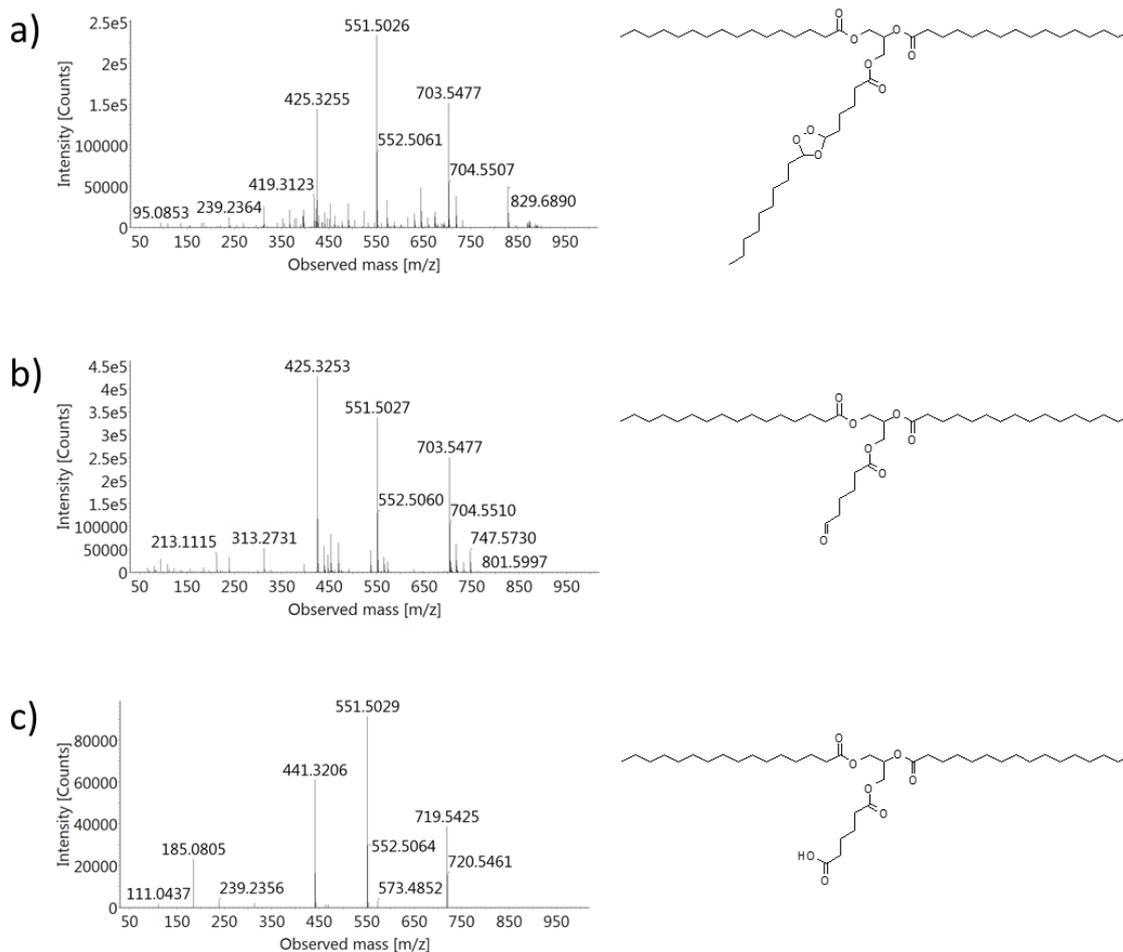


320

321 **Figure 3:** Extracted ion chromatogram of m/z 870.7392 (48:1;O₃), with inset high energy mass
322 spectra of isomers eluting at 14.81 min (top) and 14.95 min (bottom) demonstrating primary neutral
323 losses of FA 18:1;O₃ + NH₃ (m/z 523) and FA 16:1;O₃ + NH₃ (m/z 552), respectively.

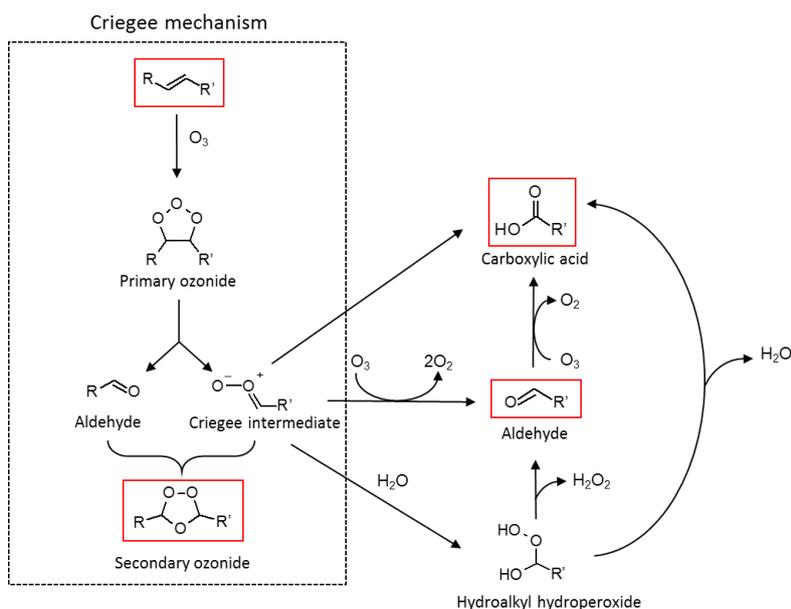
324 While sebaceous TGs consist of a number of polyunsaturated species [42, 49, 60], no ozonides were
325 identified that could be attributed to TGs with more than two double bonds in this investigation,
326 despite the detection of such lipids in the 1 day old samples. While this was initially thought to be due
327 to the neutral loss filter, as di- and triozonides tend to be cleaved at the trioxolane ring, rather than
328 undergo losses of the entire oxidised fatty acyl chain [50, 53], no m/z corresponding to [M+NH₄]⁺ ions
329 of these ozonides were found. There are several possible explanations for this. Firstly, complete
330 ozonolysis of a polyunsaturated species is progressive, with the monoozonides being the first
331 oxidation products formed, before eventual total conversion to the fully oxidised lipid [13, 50, 53]. As
332 the number of possible ozonolysis intermediates increases with the number of double bonds, it could
333 be that the division of a polyunsaturated TG across multiple oxidation products means that none of
334 them demonstrated a sufficiently high signal for detection. Secondly, environmental factors might
335 interfere with ozonide formation. It is accepted that 1,2,4-trioxolanes are produced by the reaction of
336 ozone with a double bond via the Criegee mechanism, a three step process that involves the
337 decomposition of an initial 1,2,3-trioxolane, and rearrangement into a more stable configuration [50,
338 61]. However, the Criegee intermediates, zwitterions formed during the decomposition stage, can
339 instead combine with other reactants to produce shorter chain products, form dimers, or undergo
340 isomerisation to carboxylic acids [24, 26, 50, 61-63]. In the presence of water (including humid
341 reaction conditions) carbonyl and carboxylic acid derivatives can be formed from ozonolysis products,
342 rather than a 1,2,4-trioxolane [52, 56, 64-66]. With increasing DB number, there are therefore more
343 chances for interference in the secondary ozonide formation of an unsaturated TG. Zhou *et al.*
344 observed that all trilolein ozonide signals were much lower if ozonolysis took place at 50 % relative
345 humidity (RH) compared to low humidity conditions [53]. Throughout the 28-day ageing period in this
346 study, samples in both storage conditions were exposed to increased humidity (*ca.* 35 – 40 % RH)
347 during intermittent periods.

348 A number of peaks were observed with m/z corresponding to that of a TG ozonide minus 172 Da, i.e.
349 a possible aldehyde derivative of a 1,2,3-trioxolane, where cleavage at the $\Delta 6$ position had resulted in
350 a loss of C₁₀H₂₀O₂. Sun *et al.* observed that similar decomposition products were formed during
351 ozonolysis of triolein, but did not report the fragmentation pattern of these compounds [50]. The high
352 energy mass spectra of the aldehyde derivatives observed in this study exhibited neutral losses of fatty
353 acyl groups plus ammonia, as well as a loss of 147 Da, which can be attributed to the loss of the oxoacid
354 chain (C₆H₁₀O₃) plus ammonia. Much like the TG ozonide peaks, these potential TG aldehyde
355 derivatives were present as minor peaks in the 1-day old samples, but showed significantly higher
356 relative peak signals in samples at 7 and 28 days following deposition. Similarly, other peaks were
357 tentatively identified as carboxylic acid derivatives, possibly produced through further oxidation of the
358 TG aldehyde derivatives or other intermediates produced via ozonolysis. The high energy mass spectra
359 of the carboxylic acid derivatives exhibited neutral losses of fatty acyl groups plus ammonia, as well
360 as the loss of the dicarboxylate chain (C₆H₁₀O₄) plus ammonia (163 Da). These fragmentation patterns
361 are consistent with a recent report [55]. Example high energy mass spectra and proposed structures
362 are provided in Figure 4.



363
 364 **Figure 4:** High energy mass spectra with example structures of TG 48:1 degradation products a)
 365 mono-ozonide (m/z 870), b) aldehyde derivative (m/z 698) and c) carboxylic acid derivative (m/z 714).

366 Production of decanal, oxoacids and dicarboxylic acids via the oxidation of free fatty acids in latent
 367 fingermarks has been previously described [3], and it follows that glycerides would degrade in a similar
 368 manner. While Hinners *et al.* reported both TG aldehyde and carboxylic acid derivatives from latent
 369 fingermark samples, they identified them as being products of in-source decomposition of secondary
 370 ozonides, which were not detected by the MALDI-MS method employed [55]. Possible reaction
 371 schemes for the formation of all TG ozonolysis products observed in this study are summarised in
 372 Figure 5. Further investigation is required to confirm the identities of these peaks, and whether these
 373 are indeed decomposition products formed during TG ozonolysis.



374

375 **Figure 5:** Proposed reaction mechanisms for unsaturated triglycerides with ozone. Structures
 376 observed in this study are outlined in red. Based on [53, 61-63, 65, 66].

377 TG ozonides identified at all ageing periods in this investigation are summarised in Table 6. While TG
 378 ozonides were identified in samples at all time intervals during this investigation, ozonides were
 379 identified as only minor components in 1 day old samples from 4 of the 5 donors, relative to the peak
 380 areas of the unoxidised TGs. As more recently deposited samples were not analysed, it is unclear as
 381 to whether these ozonides formed after sample collection, or whether they were already present on
 382 the donors' skin, as part of the initial fingerprint residue. It may be a continuous process, as lipid
 383 ozonolysis has been observed to take place rapidly on human skin when deliberately exposed to ozone
 384 [24, 26]. Monoozonides of TGs 44:1 – 50:1 were identified in almost every sample 7 days and older as
 385 major sample components, which is an unsurprising result given that the parent TGs comprised the
 386 most common and abundant fingerprint glycerides in the 1 day old samples. This is consistent with
 387 the significant signal reduction of unsaturated TGs described above.

CN:DBE;O _x	Number of fingerprints [n=15] (number of donors [n=5])			
	Day 1	Day 7	Day 28 (desk)	Day 28 (drawer)
40:1;O ₃	1(1)	4(2)	5(2)	3(1)
42:1;O ₃	2(1)	11(5)	7(4)	8(4)
43:1;O ₃	-	10(4)	10(4)	12(5)
44:1;O ₃	3(3)	13(5)	14(5)	14(5)
45:1;O ₃	1(1)	15(5)	15(5)	15(5)
46:1;O ₃	5(3)	15(5)	15(5)	15(5)
47:1;O ₃	7(3)	15(5)	15(5)	15(5)
48:1;O ₃	6(3)	13(5)	14(5)	15(5)
49:1;O ₃	2(1)	14(5)	13(5)	14(5)
50:1;O ₃	1(1)	12(5)	13(5)	14(5)
51:1;O ₃	-	13(5)	11(5)	11(5)
52:1;O ₃	-	9(5)	6(4)	10(4)
53:1;O ₃	-	2(1)	3(2)	-
54:1;O ₃	1(1)	3(1)	5(3)	2(1)
55:1;O ₃	-	5(3)	6(3)	6(3)

56:1;O ₃	-	4 (3)	7 (3)	6 (4)
57:1;O ₃	-	2 (2)	2 (1)	3 (2)
46:2;O ₃	4 (2)	-	-	-
47:2;O ₃	3 (2)	2 (1)	-	-
48:2;O ₃	9 (3)	1 (1)	-	-
49:2;O ₃	4 (2)	1 (1)	-	-
50:2;O ₃	5 (2)	1 (1)	-	-
44:2;O ₆	-	-	1 (1)	3 (1)
46:2;O ₆	-	4 (4)	1 (1)	1 (1)
47:2;O ₆	-	3 (2)	-	1 (1)
48:2;O ₆	-	4 (3)	1 (1)	6 (3)
49:2;O ₆	-	4 (3)	-	7 (3)
50:2;O ₆	-	2 (1)	-	3 (3)

388 **Table 6:** Qualitative profiles of triglyceride ozonides detected in fingermarks from 5 donors as a
389 function of sample age.

390 Ozonides of diunsaturated TGs were detected as both mono- and diozonides at different ageing times
391 throughout this investigation. Only monoozonides of these particular TGs were identified in samples
392 extracted 1 day after deposition. Both mono- and diozonides were identified in 7 day old samples,
393 however, monoozonides were identified less frequently compared to the 1 day old samples. Finally,
394 only diozonides were identified in samples extracted after 28 days, regardless of whether samples had
395 been stored with or without exposure to light. The concurrent detection of diozonides in samples 7
396 days and older indicates the progressive reaction of ozone with polyunsaturated TGs to their fully
397 ozonated counterparts between 7 – 28 days of ageing [13, 53]. However, the most common and
398 abundant TG ozonides identified in fingermarks at all time periods investigated through this study
399 were monoozonides of monounsaturated TGs.

400 As shown in Table 6, greater numbers of TG diozonides were identified in samples stored in the drawer
401 for 28 days, compared to those stored on the desk for the same period of time. Zhou *et al.* recently
402 reported that exposure to UV light through a closed office window led to rapid degradation of the
403 triozone of a triolein standard, whereas light-protected samples (covered in aluminium foil)
404 remained relatively stable [53]. In this study, samples stored on the desk were exposed to direct
405 sunlight on several occasions throughout this investigation; during clear mornings samples were
406 directly illuminated for several hours through an adjacent office window. However, the bulk
407 fingermark residue may physically or chemically protect ozonides from degradation [13]. Individual
408 lipid standards deposited onto a surface exhibit faster reaction rates compared to analogous lipids
409 within fingermark samples stored under the same conditions [13, 18]. Additionally, Pleik *et al.*
410 reported that ozonolysis products were not detected in fingermarks stored in a wooden desk drawer
411 for up to 63 days [13]. This could be due to the substrate used for sample collection, as filter paper
412 would absorb a greater quantity of fingermark residue than the aluminium foil used by Pleik *et al.*
413 Other possible explanations may include differences in storage conditions (i.e. materials and
414 dimensions of the drawers used in each study, as well as airtightness and external airflow) that might
415 impact the availability of ozone to react with fingermark lipids. However, it must be borne in mind
416 that only small donor populations were used in these studies, and that fingermark composition
417 exhibits high intra-donor variability. As no quantification approach was used here, it is uncertain as to
418 how light exposure, or other environmental factors, quantitatively impact the persistence of
419 fingermark TGs or their ozonides.

420

421 Conclusions

422 The degradation processes of latent fingerprint components are of great interest to forensic research,
423 from the perspective of evaluating their impact on detection, as well as the potential to estimate the
424 age of a latent fingerprint. The glyceride fraction constitutes a diverse range of structures, which
425 presents difficulties not only for their identification, but for monitoring degradation pathways and
426 identifying their products. Using UPLC-IMS-QToF-MS^E, mono- and diozonides of 23 fingerprint
427 triglycerides were identified as oxidation products formed over a 28 day period. The progressive
428 oxidation of diunsaturated triglycerides to mono- and diozonides was observed to take place in latent
429 fingerprints for the first time, through changes in qualitative profiles at 7 and 28 days after deposition.
430 Ozonolysis of latent fingerprint lipids produces a mixture of oxidation products and isomers, reflecting
431 the complexity of the initial glyceride composition. While ozonides have been previously identified in
432 latent fingerprints, this is the first time that a data-independent acquisition approach has been
433 reported, with ozonides being identified simultaneously alongside di- and triglycerides that were
434 characterised in previous work. Additionally, aldehyde and carboxylic acid derivatives were identified
435 as other possible degradation products formed through side reactions with ambient humidity.

436 The results of this investigation support the hypothesis that ozone is the major reactant involved in
437 TG degradation in latent fingerprint residue. Further work is required to determine the impact of other
438 environmental factors, such as humidity, on glyceride degradation, as well as investigating the stability
439 and possible further degradation of TG ozonides. Such fundamental information is required to enable
440 better understanding of lipid-sensitive fingerprint detection techniques, and to assess the potential
441 of ozonolysis as a basis for estimating fingerprint age.

442

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449

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