Lipid Oxidation and Degradation Products in Raw Materials: LowFat Topical SkinCare Formulations

Thomsen, Birgitte Raagaard; Taylor, Richard; Hyldig, Grethe; Blenkiron, Peter; Jacobsen, Charlotte

Published in:
J A O C S

Link to article, DOI:
[10.1002/aocs.12087](https://doi.org/10.1002/aocs.12087)

Publication date:
2018

Document Version
Peer reviewed version

[Link back to DTU Orbit](#)

Citation (APA):
Thomsen, B. R., Taylor, R., Hyldig, G., Blenkiron, P., & Jacobsen, C. (2018). Lipid Oxidation and Degradation Products in Raw Materials: LowFat Topical SkinCare Formulations. *J A O C S*, 95(7), 853-864.
<https://doi.org/10.1002/aocs.12087>

General rights

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

1 **Lipid oxidation and degradation products in raw materials: Low fat topical skin care**
2 **formulations**

3 Authors: Thomsen, B.R.¹, Taylor, R.², Hyldig, G.¹, Blenkiron, P.² & Jacobsen, C.^{1*}

4 ¹National Food Institute, Division of Food Technology, Technical University of Denmark, Lyngby,
5 DK, ²GlaxoSmithKline, Brentford, UK

6 **Abstract**

7 Topical skin formulations with a lipid content below 15% were stored for six months at 5 °C, 20 °C,
8 or 40 °C or for 2 weeks at 50 °C in darkness or at 20 °C with exposure to light for six months . The
9 volatile lipid oxidation compounds formed during this storage were compared to those formed in
10 the raw materials during three months of accelerated stability storage at 40 °C for 3 months. The
11 volatile compounds were collected by dynamic headspace and analysed by GC-MS.

12 It was possible to link eight out of nine volatile compounds detected during storage of topical skin
13 formulations to the raw materials. In addition, a possible link between the appearance of butane
14 nitrile and the decomposition of an initiator used for polyacrylate crosspolymer-6 production was
15 observed. The polymer may originate from texture modifiers added to the topical skin formulation
16 or from plastics used for packaging of topical skin formulations.

17 Furthermore, six well-known lipid oxidation and non-enzymatic browning products were suggested
18 to originate from the two raw materials, tricaprylin/tricaprin and coconut oil.

19
20
21
22
23
24
25
26
27
28
29
30
31

32 **Introduction**

33 In earlier studies, it was clearly demonstrated that lipid oxidation can occur in topical skin
34 formulations with either high or low lipid content and that their sensory quality can be affected by
35 lipid oxidation as changes in both odour and colour were observed [1-6]. It is tempting to assume
36 that the extent of lipid oxidation will decrease with decreasing lipid content. However, this may not
37 always be the case in “real” topical skin formulations because factors other than the absolute lipid
38 content may be more important. Similar to other emulsions, topical skin formulations consist of
39 three phases: the lipid and aqueous phase and an interface. Lipid oxidation is affected by
40 partitioning and diffusion of anti-oxidants and pro-oxidants in all three phases and this can
41 significantly influence lipid oxidation. Physical factors such as viscosity and droplet size may affect
42 diffusion of pro-oxidants and, thereby, increase or decrease lipid oxidation [7].

43 Topical skin care formulations are complex systems consisting of many different ingredients, which
44 may affect lipid oxidation positively or negatively. In addition, interactions between ingredients
45 could influence lipid oxidation as also observed for simple emulsions [8]. However, only a limited
46 number of studies on this topic are available in the literature. An earlier study of the skin
47 conditioning raw material, coconut oil, showed that its peroxide value (PV) increased from
48 approximately 2 to 65 meq/kg during 42 days of storage at 60 °C [9]. PV measures the primary
49 oxidation products, which are odourless. Hence, PV cannot be used to assess odour changes in raw
50 materials as a result of lipid oxidation. Odour changes are caused by secondary volatile oxidation
51 products which were not measured in the above mentioned study.

52 Data on the quality of other raw materials used in topical skin care formulations are available in the
53 literature [3, 9, 10]. However, the quality may vary widely depending on manufacturing process.
54 This was shown in a more recent study, which compared the quality of coconut oils produced in
55 India [10]. The authors concluded that the quality varied widely depending on crop quality and
56 production method. They analysed three types of coconut oil; virgin coconut oil from wet mature
57 coconut, and refined, bleached, and deodorized and unrefined coconut oil prepared from copra.
58 Furthermore, they measured oxidative status by PV, which fluctuated from 0.0 to 2.7 meq/kg
59 depending on manufacturer. In addition, the colour (0.00 – 2.7 Lovibond unit), free fatty acid (0.01
60 – 2.02 %), polyunsaturated fatty acid (0.13 – 1.57 %), monounsaturated fatty acid (3.31 – 5.23 %),
61 saponification value (239.9 – 260.2) also varied widely between the production methods. The
62 conclusion was that virgin coconut oil had the best quality independent of manufacturer [10]. These

63 studies support the notion that raw materials used for topical skin formulations may vary widely in
64 quality because of their manufacturing process and that attention therefore must be paid to this fact
65 when selecting raw materials for the manufacture of such products.

66 In an earlier study, we investigated lipid oxidation in raw materials used for production of a topical
67 skin formulation with a high lipid content. We found that the most dominating volatile compounds
68 clearly originated from specific raw materials [1].

69 Several studies have explored the effect of lipid content on the oxidative stability in oil-in-water
70 emulsions. One of these studies investigated the effect of pH and emulsifier type in two emulsions
71 with a lipid concentration of 5 % and 70 %, respectively [11, 12]. In general, the study showed that
72 the oxidative stability of the 5 % lipid emulsion was lower than the oxidative stability of the 70 %
73 lipid emulsion. The lower oxidative stability was independent of emulsifier type. The findings were
74 surprising as more oxygen can be dissolved in oil than in water. It was suggested that the
75 accessibility to direct interaction of lipid hydroperoxides with the prooxidant trace metal ions in the
76 aqueous phase was lower in the emulsions with high lipid content [11, 12, 13]. Aligned with our
77 previous studies [11, 12], another study compared the oxidative stability of 10 % vs 30 % oil-in-
78 water emulsions [13]. The oxidative stability was assessed by PV and anisidine value (AV) of the
79 emulsions. The PV and AV were significantly affected by the lipid content, the 10 % emulsions had
80 significantly higher amounts of hydroperoxides and aldehydes after 15 days storage compared to
81 the 30 % emulsions. Overall, a decrease in the lipid content resulted in increased lipid oxidation
82 [13]. The lower oxidative stability in these three studies may be related to low concentration of oil
83 and thereby higher concentration of water that contains the prooxidant trace metal ions. Trace metal
84 ions are well-known initiators of lipid oxidation in the initiation stage but also in the decomposition
85 of hydroperoxides to secondary oxidation products. However, other factors than the oil content and
86 presence of trace metal ions can also affect the oxidative stability. For example differences in oil
87 droplet size could have affected the oxidative stability.

88 Before we are able to understand interactions between the raw materials in topical skin
89 formulations, the oxidative status of the raw materials must be explored. The hypothesis of this
90 study is therefore that oxidation occurs to a higher extent in low fat than in high fat topical skin care
91 formulations and that it is possible to link the volatile compounds to the raw material(s) used in the
92 formulations.

93 The purpose of this study was thus to measure lipid oxidation and oxidative degradation products in
94 topical skin formulations with a lipid content below 15 % as well as in selected raw materials. A
95 second aim was to investigate the link between volatile compounds affecting quality in topical skin
96 formulations to the presence and formation of the same volatile compounds in raw material(s). In
97 addition, we aimed at obtaining an understanding of the mechanism leading to the formation of
98 certain volatile compounds. We used refined coconut oil and medium chain tricaprylin and tricaprin
99 lipid sources in the formulations.

100 Lipid oxidation was accelerated by increasing temperature or light exposure to more quickly
101 generate potential volatile compounds.

102 **Materials**

103 Prototype skin formulations

104 The topical skin care formulations used in this study were produced by GlaxoSmithKline
105 (Brentford, United Kingdom). Two types of topical skin formulations were included in this study
106 due to their different purposes and therefore different raw materials; 1) a prototype cleansing
107 formulation (PCF) that contains rinsing agents to clean the skin. 2) a prototype serum formulation
108 (PSF) that has a higher concentration of performance ingredients than other topical skin
109 formulations with a low lipid content. It is used for targeting specific skin care concerns. The PCF
110 contained several raw materials including glycerine, tricaprylin and tricaprin, coconut oil, lecithin
111 and polyacrylate crosspolymer-6. The PSF contained several raw materials including glycerine and
112 lecithin. Manufacturing protocols for the formulations are proprietary information.

113 Raw materials

114 Tricaprylin and tricaprin having the commercial name “Caprylic/capric triglycerides” 100 % (BASF
115 SE, Ludwigshafen, Germany), Glycerine 99.5 % (Croda Europe Ltd, East Yorkshire, England),
116 Coconut oil 100 % (Henry Lamotte oils, Bremen, Germany), Lecithin 88.2 % (Lipoid,
117 Ludwigshafen, Germany), Polyacrylate crosspolymer-6 92-100 % (Seppic, Paris, France)

118 **Methods**

119 Storage experiment

120 PCF (in full 200 ml plastic bottles) and PSF (in full 30 ml plastic bottles) were stored for six months
121 at 5 °C, 20 °C, and 20 °C with exposure to artificial light (approximately 3500 lx), 40 °C, and for 2

122 weeks at 50 °C with sampling points after 0, ½ (only 50 °C), 1, 2, 3 and 6 months. Individual
123 containers were removed at each time point: one bottle of PCF and 3 bottles of PSF.

124 Raw materials were stored at 40 °C for 3 months. Samples were taken each month (0, 1, 2 and 3
125 months). This storage condition was used to accelerate degradation and oxidation fast to reveal
126 which volatile compounds that a certain raw material may give rise to. Furthermore, the
127 concentrations in raw material and finished products are not to be compared directly

128 The samples were stored at 5 °C until PV and GC-MS analysis. The product odor was assessed by
129 an expert panel consisting of scientists in the R&D department at GSK at the end of the storage
130 period. The scientists have expertise in using the degree of Difference (DOD) scale which they use
131 at GSK to assess odor changes during storage. In this method, the sample odour was graded versus
132 a reference. All samples are ranked from one to five. All samples ranked below 4 are within
133 “product range”.

134

135 Oil extraction

136 Oil was extracted from PCF and PSF with Bligh and Dyer method using a reduced amount of
137 solvent [14, 15]. The method is described in more details in [1]. In brief, the water-soluble parts
138 were separated from the lipid soluble parts by addition of chloroform, water and methanol followed
139 by centrifugation. The lipid phase was used as starting material for PV analysis and determination
140 of fatty acid composition.

141 Peroxide Value

142 PV was determined spectrophotometrically at 500 nm using the IDF method [16].

143 Quantification of volatile compounds

144 The volatile compounds were selected based on a prescreening, which considered their presence
145 and increasing concentration in PCF and PSF during storage. The volatile compounds that appeared
146 in PCF and PSF are different because they are produced from different raw materials.

147 *Purge and trap on PCF*

148 Extraction of volatile compounds and GC-MS analyses were performed as described by Thomsen et
149 al. [17] for emulsions. In brief, the volatile compounds were released by continuously disturbing the
150 equilibrium between the sample and headspace by purging nitrogen directly through the sample.
151 The volatile compounds released from the sample were absorbed on a tube containing Tenax GR.
152 After collection, the Tenax tube was manually inserted into an automatic thermal desorption unit
153 (ATD 400, Perkin Elmer, Norwalk, CT, USA), which transferred the volatile compounds from the
154 Tenax tube to a focusing cold trap (-30 °C). Thereafter, the volatile compounds were transferred to
155 the GC (Agilent 5890 IIA model Palo Alto, CA, USA) equipped with a DB1701 column (30 m × ID
156 0.25 mm × 0.5 µm film thickness, J&W Scientific, Folsom, CA, USA) using helium gas flow (1.3
157 mL/ min). The GC was connected to MS HP 5972 (Palo Alto, CA, USA) for analysis.

158 *TDU/DHS on PSF and raw materials*

159 Extraction of volatile compounds and GC-MS analyses were performed automatically using thermal
160 desorption unit/dynamic headspace (DTU/DHS) as described by Thomsen et al. [17] with the
161 following modifications for sample preparation, collection and water evaporation (Table 1). The
162 extraction modification was performed in order to avoid contamination of the system and to remove
163 water residues. Briefly, volatile compounds were automatically collected by purging the headspace
164 (and not through the sample) followed by trapping the volatile compounds on the adsorbent tube
165 using the Gerstel Tenax GR 300 tubes in a dynamic headspace station (Gerstel GmbH & Co. KG.,
166 Mülheim an der Ruhr, Germany). Then, the absorbent tube was automatically transferred by a
167 thermal desorption unit/CIS (Gerstel GmbH & Co. KG., Mülheim an der Ruhr, Germany) into the
168 GC 6890N Series –MS 5973 inert mass-selective detector (Agilent Technologies, Santa Clara,
169 USA).

170 *GC temperature program and MS settings for both purge and trap, and DTU/DHS*

171 GC temperature-program: initial 45 °C for 5 min, 5 °/min til 90 °C, 4 °C/min to 220 °C and held for
172 4 min. The MS settings: electron ionization mode, 70 eV, mass to charge ratio (m/z) scan between
173 30 and 250.

174 Fatty acid methyl esters (FAME)

175 Fatty acid compositions of coconut oil, tricaprylin and tricaprin were determined in accordance with
176 the method by Safafar *et al.* [18]. The analysis was conducted on 0.3 g of oil. Then, to the oil was
177 added 100 µl internal standard 23:0 together with 200 µl heptane with BHT, 100 µl toluene and 1ml
178 borontrifluoride in methanol. Samples and reagents were mixed and methylation was performed in a
179 microwave oven at 100 °C for 5 min (Microwave 3000 SOLV, Anton Paar, Ashland, VA, USA)
180 and the methylated sample was cooled down to room temperature. Then, to the methylated sample
181 was added 1ml saturated NaCl and 0.7 ml heptane with BHT. Phase separation occurred, and the
182 lipid/heptane phase of the methylated sample was analysed with Agilent 7890A GC (Agilent
183 Technologies, Palo Alto, CA, USA) equipped with a DB-WAX fused silica capillary column (10
184 m×0.1 mm, 0.1 µm; Agilent Technologies, Palo Alto, CA, USA), using helium as carrier gas and a
185 flame ionization detector. The GC temperature program: initial 160 °C, 10.6 °C/min until 200°C
186 and held for 0.3 min, 10.6°C/min to 220°C and held for 1 min, and 10.6°C/min to 240°C and held
187 for 3.8 min. The individual fatty acids were identified by matching their retention times to those of
188 authentic standards. The result was expressed as area % of total fatty acids having a chain length
189 between C8-C24, however the values reported below C14 are estimations. Only individual fatty
190 acids present above 0.5 % was included.

191 Statistics analysis

192 A two-way analysis of variance followed by a Bonferroni multiple comparison test was employed
193 to evaluate significant changes in PV (duplicates) and volatile oxidation products (triplicates)
194 during storage. The calculation was conducted using Graph pad prism version 6 (graph pad, La
195 Jolla, USA).

196

197

198 **Results and discussion**

199 Lipid oxidation in products

200 In PCF, PV was low initially and remained below 1 meq/kg during six months at 5 °C, 20 °C, 40 °C
201 and during two weeks at 50 °C (Figure 1A). It was not surprising that exposure to light increased
202 PV significantly to 20 meq/kg after two months of storage. This pattern was observed in other
203 studies [2, 4, 6]. GC-MS analysis of the volatile compounds confirmed that lipid oxidation only
204 occurred to a limited extent. Several volatile compounds were present in low concentrations (below

205 odour detection threshold (ODT) value in water) and did not show any clear pattern during six
206 months (data not shown). This was the case for 2-ethyl furan, pentanal, 1-penten-3-ol, 3-methyl-1-
207 butanol, hexanal, 1-hexanol, heptanal, 1-heptanol, 2-ethyl-1-hexanol, 1-octanol, nonanal and
208 decanal.

209 Even though lipid oxidation only occurred to a low extent, the concentrations of six volatile
210 compounds increased during storage namely butanal, butanenitrile, 1-pentanol (Figure 1B-D),
211 pentanenitrile, hexanenitrile and octanenitrile (data not shown). Butanal was not present initially
212 and increased only slightly but significant during six months storage to 11-12.1 ng/g at 5 °C, 20 °C
213 and 20 °C with exposure to light (Figure 1B), respectively. When exposed to 40 °C a significantly
214 higher concentration was obtained after six months (16.5 ng/g). 1-Pentanol increased significantly
215 to approximately 7 ng/g at all conditions after 6 months storage (Figure 1D). Butanal and 1-
216 pentanol are well-known lipid oxidation products [19, 20, 21]. In an earlier study, we determined
217 ODT values for volatile oxidation products, which increased during storage in a prototype cleansing
218 formulation. In general, we found that ODT values in a prototype cleansing formulation were above
219 70 ng/g [6]. The cleansing formulation in this study is a matrix comparable to the cleansing
220 formulation used for the ODT study, but with some small differences, which did not disqualify the
221 ODT values previously determined from being used in the present study.. Therefore, butanal and 1-
222 pentanol most likely did not affect product odour as individual compounds, but they may contribute
223 to a cocktail effect when present together with other volatile compounds.

224 Butanenitrile, pentanenitrile, hexanenitrile and octanenitrile were reported to be present in topical
225 skin formulations [2] but the mechanism of their formation during storage was not explained.
226 Butanenitrile has been described as having a bitter almond-like odour [22, 23]. The ODT value in
227 water was 32 ng/g for butanenitrile [24]. The concentration of butanenitrile, pentanenitrile,
228 hexanenitrile and octanenitrile increased significantly during storage at 20 °C with exposure to light
229 and 40 °C. However, the concentrations were below 70 ng/g, they are not expected to affect odour
230 as individual compounds.

231

232 Similar to PCF, PSF was also selected as a representative of topical skin formulations with low lipid
233 content. PSF contains a higher number of skin conditioning raw materials compared to PCF.

234 For PSF, initially PV was 0.25 meq/kg and it remained below 0.3 meq/kg at 5 °C. It increased
 235 significantly after 6 months' storage to 1.0 meq/kg, 1.3 meq/kg and 2.5 meq/kg at the storage
 236 conditions 20 °C, 20 °C with exposure to light and 40 °C, respectively (Figure 2A). Again, the low
 237 PV may be related to a fast conversion of hydroperoxides to secondary volatile oxidation products.
 238 After 3 months' storage, most volatile oxidation compounds seemed to be formed almost
 239 simultaneously with the peroxides indicating that there was a lag period before oxidation took off
 240 after 2 months storage (Figure 2). Concentrations of several aldehydes increased in PSF during
 241 storage: butanal, pentanal, hexanal and benzaldehyde. Butanal, pentanal and hexanal are all well-
 242 known lipid oxidation products, whereas benzaldehyde has been suggested to arise from non-
 243 enzymatic browning reactions [19, 20, 25]. In addition to the aldehydes, the concentration of two
 244 ketones (2-pentanone and 2-hexanone) and one alcohol (1-pentanol) increased as well.

245 For the two short-chained aldehydes, butanal and pentanal, concentrations were initially low but
 246 increased significantly during storage at all conditions. After 6 months of storage, their
 247 concentrations increased significantly and above the ODT values at 130 ± 10 ng/g and 100 ± 6 ng/g
 248 for butanal and pentanal obtained in topical skin formulations with a low lipid content [6]. The
 249 ODT values were exceeded for PSF stored at 20 °C (only for pentanal), 20 °C with exposure to light
 250 and 40 °C. For butanal, the concentration increased to 154 ng/g and 141 ng/g in PSF stored during 6
 251 months at 20 °C with exposure to light and 40 °C, respectively (Figure 2B). This concentration of
 252 butanal can affect the odour to become more cheese-like and citrus sour [6]. For pentanal, the
 253 concentration increased to 119 ng/g, 184 ng/g and 185 ng/g when stored during 6 months at 20 °C,
 254 20 °C with exposure to light and 40 °C, respectively (Figure 2D). This concentration of butanal can
 255 affect the odour to become more green and acidic milk-like [6].

256 In contrast to the large increases observed in the concentrations of short chained aldehydes,
 257 concentrations of the two aldehydes with a longer chain, hexanal and benzaldehyde, only increased
 258 slightly to 69 ng/g (but significantly after 6 months at 20 °C with exposure to light and 40 °C) and
 259 17 ng/g (Figure 2F and 2H). No significant increases were observed for the two ketones, 2-
 260 pentanone and 2-hexanone, for which their concentrations only increased slightly to 13 ng/g and 15
 261 ng/g (Figure 2C and 2E). These low concentrations are not expected to affect product odour as
 262 individual compounds although they may contribute to a cocktail effect.

263 The alcohol, 1-octanol increased significantly during storage at 40 °C to 1803 ng/g after 6 months'
 264 storage. At this high concentration, it is expected to affect product odour. At the other storage

conditions, 1-octanol also increased significantly after 6 months' storage to 102 ng/g, 279 ng/g and 222 ng/g at 20 °C, 20 °C with exposure to light and 40 °C, respectively (Figure 2G). The exact ODT value for 1-octanol in topical skin formulation has not been determined, but the ODT value for 1-heptanol has been measured in topical skin formulation and was found to be 170 ± 23 ng/g [6]. The volatility of 1-octanol is expected to be lower than 1-heptanol. Therefore, a slightly higher ODT value may be expected for 1-octanol than for 1-heptanol. Nevertheless, it is likely that the observed concentrations after 6 months (279 ng/g, 222 ng/g and 1083 ng/g) may affect product odour.

Comparison of PV results with PV data obtained in our earlier study [1], showed that the oxidative stability was lower for topical skin formulations having a low lipid content than in topical skin formulations with a high lipid content. The PV was up to 21.9 meq/kg and 1.44 meq/kg in topical skin formulations having a low and high lipid content, respectively. However, the concentration of volatile oxidation and degradation products revealed that the oxidative stability of topical skin formulations with a high lipid content was lowest. The same pattern was observed in this study namely that a low PV was related to a high concentration of volatile compounds, but a high PV did not result in a high concentration of volatile compounds. Consequently, no direct relationship was observed between PV and volatile compounds neither in the present study nor in the previous study. The concentration of butanal increased to 154.35 ng/g and 408.72 ng/g in topical skin formulations having a low and high lipid content, respectively. The higher PV for topical skin formulations with a low lipid content may be related to a faster conversion from primary to secondary oxidation products. The hypothesis that the low fat topical skin care formulations would have a lower oxidative stability than the high fat products could thereby not be confirmed. Studies in simple emulsions [11, 12, 13] obtained the opposite result namely that decreasing lipid content increased oxidation. The reason for these contradicting results may be related to effects on lipid oxidation from the numerous raw materials used for topical skin formulations e.g. addition of polymers, which may make it difficult to compare the effect of the lipid content in different types of products. Therefore, more studies are needed to investigate this issue.

Lipid oxidation in selected raw materials

A broad screening for volatile compounds in mainly lipid containing raw materials was conducted. The concentration of product relevant to volatile compounds increased notably in five of the raw materials used for the prototype skin formulations during 3 months' storage, namely, glycerine and

296 lecithin applied in PCF and PSF, and tricaprylin and tricaprin, coconut oil and polyacrylate
297 crosspolymer-6 for PCF only.

298 The main focus was on volatile compounds initially present and also appearing during storage both
299 in topical skin formulations and raw materials. An explanation to the appearance of the volatile
300 compounds in the raw material is suggested based on fatty acid composition (degree of
301 unsaturation) and other studies reported in literature.

302 The raw materials; tricaprylin and tricaprin, glycerine and coconut oil contained several aldehydes
303 (Figure 3). The concentration of volatile compounds increased particularly in tricaprylin and
304 tricaprin, and coconut oil during accelerated storage at 40 °C for 3 months.

305

306 The increasing concentration of butanal in both prototype skin formulations may be related to two
307 raw materials, tricaprylin and tricaprin and glycerine. The raw material, tricaprylin and tricaprin,
308 also contained 1-pentanol and 2-pentanone after accelerated storage. Therefore, the increase in the
309 concentration of 1-pentanol in PCF may be related to this raw material.

310 The three aldehydes, pentanal, hexanal and benzaldehyde, and one ketone, 2-hexanone, originated
311 partly from three raw materials, tricaprylin and tricaprin, glycerine, and coconut oil. Several other
312 volatile compounds increased in these raw materials but not in the products, namely 3-
313 methylbutanal, 3-methyl-1-butanol, 2-heptanone, heptanal, octanal and nonanal. We hypothesize
314 that this could be because the skin care product matrix highly influenced the release of volatile
315 compounds. Furthermore, the concentration of the volatile compound detected in the neat raw
316 material may be below the detection limit for GC-MS method when the raw material is mixed with
317 other ingredients in the skin care product. However, 6 out of 9 volatile compounds that increased
318 during storage in PCF also appeared and increased in the raw materials.

319 The increasing concentration of volatile compounds in tricaprylin and tricaprin can be related to its
320 fatty acid composition. tricaprylin and tricaprin mainly contained fatty acids with a shorter chain
321 length than C14. It contained the saturated fatty acids (>99 %; 57.02 % of 8:0 and 42.30 % of 10:0)
322 and unsaturated fatty acids (< 0.5 %; 16:1 and 16:2). Even though the degree of unsaturation was
323 low, tricaprylin and tricaprin oxidized to a large extent during accelerated storage. This may be due
324 to the fact that monounsaturated fatty acids can undergo autooxidation at elevated temperature.

325 The humectant raw material, glycerine was a relatively stable raw material. As described previously
326 [1], glycerine can oxidize to aldehydes such as glyceraldehyde, which may react with other

327 molecules through the mechanism described by Jungermann and Sonntag [26]. However, impurities
328 in the raw material (0.5 %) may also contribute to the volatile compounds developing during
329 accelerated storage.

330 The other raw material for which the concentration of volatile compounds increased during storage
331 was coconut oil. Again, it can be explained by its fatty acid composition. Coconut oil mainly
332 contained the saturated fatty acids (> 90 %; 7.65 % of 8:0, 5.81 % of 10:0, 45.68 % of 12:0, 18.29
333 % of 14:0, 9.89 % of 16:0, 2.87 % of 18:0). However, it also contained unsaturated fatty acids;
334 mono-unsaturated (> 7 %; 7.30 % of 18:1 n-9) and polyunsaturated (< 2 %; 1.83 % of 18:2 n-6).
335 The polyunsaturated fatty acids are highly susceptible to autoxidation, which may explain the large
336 increase observed in the concentration of volatile compounds.

337
338 In addition to the lipid containing raw materials, other raw materials, which were present in a
339 concentration above 1 %, were also screened for volatile compounds during accelerated storage.
340 Amongst those, lecithin and polyacrylate crosspolymer-6 had increasing concentrations of at least 6
341 of the 9 products relevant volatile compounds (Figure 4).

342 After 3 months of accelerated storage, a broad variety of volatile compounds appeared in both raw
343 materials. In lecithin and polyacrylate crosspolymer-6, the concentration of butanal, 2-pentanone
344 (only lecithin), pentanal, 2-hexanone, hexanal and benzaldehyde increased. Therefore, these raw
345 materials may partly contribute to the increasing concentrations observed in the topical skin
346 formulations. Several other volatile compounds increased in the texture modifying raw materials,
347 but not in the prototype skin formulations: 3-methylbutanal, 2-heptanone, heptanal, octanal and
348 nonanal.

349 None of the raw materials contained butane, pentane, hexane or octanenitrile. However, a nitrile
350 containing compound was found in polyacrylate crosspolymer-6, namely tetramethylbutanedinitrile.
351 It was therefore speculated that butanenitrile appearing in the PCF during storage may be related to
352 decomposition of the nitrile containing impurities in polyacrylate crosspolymer-6.

353 A literature search showed that other authors have studied the formation of butanenitrile (and other
354 nitrile containing compounds), but most of the reactions suggested to lead to the formation of
355 butanenitrile require high temperature above 176.85 °C [27]. The high temperature reaction
356 conditions required exclude the reactions from taking place in this study as butanenitrile is
357 appearing at low tempeture at room temperature. However, an alternative reaction route to
358 butanenitrile may have occurred. Tetramethylbutanedinitrile has been suggested by other authors as

359 a by-product from polymer and plastic production. In polymer and plastic production,
360 azobisisobutyronitrile (AIBN) is often used as an initiator of polymerisation. After AIBN has
361 fulfilled its purpose in polymer and plastic production, it decomposes to form 2-cyanoprop-2-yl
362 radicals or/and tetramethylbutanedinitrile as by-products (Figure 5). Several authors have reported
363 isobutanenitrile as a secondary by-product from AIBN, but no authors have reported that they have
364 detected butanenitrile [28, 29].
365 Since the formation of isobutanenitrile requires less energy than the formation of butanenitrile [30],
366 it is surprising that this compound could not be detected, whereas we were able to detect
367 butanenitrile. Another possibility is that butane, pentane, hexane and octanenitriles are migrants
368 from plastic packaging. Since, butane, pentane, hexane and octanenitriles appear in prototype skin
369 formulations stored in both plastic and glass packaging, migration does, however, not seem to be a
370 plausible explanation. More studies are needed to fully understand the reaction routes leading to the
371 detected nitriles.

372 Linking volatile oxidation products in PCF/ PSF and raw materials together

373 The volatile compounds both present in the prototype skin formulations, PCF and PSF, and in raw
374 materials are summarized in Table 2. In brief, the increasing concentration of butanal in PCF and,
375 especially, PSF during storage may originate from all the selected raw materials except coconut oil.
376 However, the concentrations were conspicuously higher and above ODT value after accelerated
377 storage in tricaprylin and tricaprin, and polyacrylate crosspolymer-6. Therefore, it is most likely that
378 butanal originated from these two raw materials.

379 It is not surprising that pentanal increased in all raw materials. This was also the case in our
380 previous study [1]. The concentration was above ODT value in all raw materials except glycerine
381 after 3 months accelerated storage. The volatile aldehyde in PSF, hexanal, was present in all raw
382 materials after 3 months accelerated storage. Especially, tricaprylin and tricaprin, and coconut oil
383 had high concentrations of hexanal at 1681 ng/g and 3976 ng/g. Benzaldehyde increased in PSF to
384 17 ng/g after 6 months' storage. It was possible to link benzaldehyde to all raw materials.

385 The appearance of butanenitrile was surprising; it is usually not observed in studies of lipid
386 oxidation. A likely explanation to its appearance has been suggested, but more studies are needed to
387 identify the exact route of formation from impurities in polyacrylate crosspolymer-6.

388 The alcohol 1-pentanol increased in one raw material, namely tricaprylin and tricaprin. The other
389 alcohol that increased in PCF, 1-octanol, was not linked to any of the raw materials. As observed
390 previously [1], the two ketones, 2-pentanone and 2-hexanone, were only present in low
391 concentrations in both PSF and raw materials.

392 Despite the product odour changing during storage in topical skin formulation, the product odour
393 was still deemed within product range by an expert panel.

394 Therefore, it was possible to link eight out of nine volatile compounds found in the prototype skin
395 formulations to raw materials. GSK Toxicology group has assessed the human safety impact of the
396 volatiles included in this study. At the determined levels these substances do not raise any
397 toxicological concern, neither locally or systemically.

398 **Conclusion**

399 This study explored lipid oxidation and oxidative degradation in two topical skin formulations (PCF
400 and PSF) containing a low lipid content. In an earlier study, we investigated lipid oxidation and
401 oxidative degradation in a topical skin formulation containing a high lipid content. Comparison of
402 these two studies revealed that the oxidative stability measured by PV decreased with decreasing
403 lipid content for topical skin formulations. However, the opposite was observed for the
404 concentration of volatile oxidation and degradation products. Thus, the concentration of the volatile
405 compounds was higher in the topical skin formulations with a high lipid content than in the topical
406 skin formulations with a low lipid content. These results are contrary to those for simple emulsions.
407 The higher stability of the topical skin formulations with a low lipid content may be related to their
408 high complexity due to the large number of raw materials, which can affect lipid oxidation such as
409 Polyacrylate crosspolymer-6. However, more studies are needed to investigate this difference
410 between simple emulsions and topical skin formulations.

411 Similar to our previous findings for topical skin formulations with a high level of lipids, several
412 secondary volatile oxidation products were present initially and more were formed during 6 months
413 of storage.

414 Selected raw materials were explored in order to link volatile compounds affecting the quality in the
415 topical skin formulation to raw material(s) and eight out of nine volatile compounds found in
416 topical skin formulations could be linked to their presence in raw materials. Thus, well-known lipid
417 oxidation products and non-enzymatic browning products found in PSF and PCF were suggested to

originate from tricaprylin and tricaprin, and in particular coconut oil because of its unsaturated nature. Butanenitrile appeared during storage in PCF. This compound has not been reported in other lipid oxidation studies either in model emulsions, food emulsions or in topical skin formulations. Since the concentration of butanenitrile was low it most likely did not affect product odour, but it is still important to explore the mechanism behind its formation. A possible link between butanenitrile and the decomposition of the initiator used for production of polyacrylate crosspolymer-6 was identified. However, more studies are needed to determine the exact reaction route from this ingredient to butanenitrile.

427

428 Literature

- 429 [1] Thomsen, B. R., Taylor, R., Madsen, R., Hyldig, G., Blenkiron, P. and Jacobsen, C.
430 Investigation of lipid oxidation in the raw materials of a topical skin formulation: A topical skin
431 formulation containing a high lipid content. Submitted *J. Am. Oil Chem. Soc* 2017.
- 432 [2] Thomsen, B. R., Horn, A. F., Hyldig, G., Taylor, R., Blenkiron, P. and Jacobsen, C.
433 Investigation of lipid oxidation in high- and low-lipid-containing topical skin formulations.
434 Submitted *J. Am. Oil Chem. Soc* 2017.
- 435 [3] Thanonkaew, A., Wongyai, S., Decker, E. A. and McClements, D. J. Formation, antioxidant
436 property and oxidative stability of cold pressed rice bran oil emulsion. *J. Food Sci. Technol.* 2015,
437 52, 6520–6528.
- 438 [4] Poyato, C., Thomsen, B. R., Hermund, D. B., Ansorena, D., Astiasaran, I., Jonsdottir, R.,
439 Kristinsson, H. G. and Jacobsen, C. Antioxidant effect of water and acetone extracts of *Fucus*
440 *vesiculosus* on oxidative stability of skin care emulsions. *Eur. J. Lipid Sci. Technol.* 2017, 119,
441 1600072(1-11).
- 442 [5] Malinowska, P., Gliszczynska-Swiglo, A. and Szymusiak, H. Protective effect of commercial
443 acerola, willow and rose extracts against oxidation of cosmetic emulsions containing wheat germ
444 oil. *Eur. J. Lipid Sci. Technol.* 2014, 116, 1553-1562.
- 445 [6] Thomsen, B. R., Hyldig, G., Taylor, R., Blenkiron, P. and Jacobsen, C. Threshold values of
446 volatile compounds in rich day cream and cleanser. Submitted *Eur. J. Lipid Sci. Technol.* 2017.
- 447 [7] Waraho T., McClements D. J., and Decker E. A. Mechanisms of lipid oxidation in food dispersions.
448 *Trends Food Sci. Technol.* 2011, 22, 3-13.

449 [8] Sørensen, A. D., Haahr, A. M., Becker, E. M., Skibsted, L. H., Bergenståhl, B., Nilsson, L. and
 450 Jacobsen, C. Interactions between iron, phenolic compounds, emulsifiers, and pH in omega-3-
 451 enriched oil-in-water emulsions. *J. Agric. Food Chem.* 2008, *56*, 1740-1750.

452 [9] Mannekote, J. K. and Kailas, S. V. Studies on boundary lubrication properties of oxidised
 453 coconut and soy bean oils. *LUBR SCI* 2009, *21*, 355-365.

454 [10] Kumar, P. K. P. and Krishna, A. G. G. Physicochemical characteristics of commercial coconut
 455 oils produced in India. *Grasas Aceites* 2015, *66*, 1-11.

456 [11] Nielsen, N. S., Horn, A. F. and Jacobsen, C. Effect of emulsifier type, pH and iron on oxidative
 457 stability of 5% fish oil - in - water emulsions. *Eur. J. Lipid Sci. Technol.* 2013, *115*, 874-889.

458 [12] Horn, A. F., Nielsen, N. S., Andersen, U., Søgaaard, L. H., Horsewell, A. and Jacobsen, C.
 459 Oxidative stability of 70% fish oil-in-water emulsions: Impact of emulsifiers and pH. *Eur. J. Lipid*
 460 *Sci. Technol.* 2011, *113*, 1243-1257.

461 [13] Osborn, H. T. and Akoh, C. C. Effect of emulsifier type, droplet size, and oil concentration on
 462 lipid oxidation in structured lipid-based oil-in-water emulsions. *Food Chem.* 2004, *84*, 451-456.

463 [14] Bligh, E. G. and Dyer, W. J. A rapid method of total lipid extraction and purification. *Can. J.*
 464 *Biochem. Physiol.* 1959, *37*, 911-917.

465 [15] Iverson, S. J., Lang, S. L. C. and Coope, M. H. Comparison of the Bligh and Dyer and Folch
 466 methods for total lipid determination in a broad range of marine tissue. *Lipids* 2001, *36*, 1283-1287.

467 [16] International IDF Standard 74 A. Milk and milk products: determination of the iron content,
 468 *International Dairy Federation* 1991, Brussels.

469 [17] Thomsen, B. R., Yesiltas, B., Sørensen, A.-D. M., Hermund, D. B., Glastrup, J. and Jacobsen,
 470 C. Comparison of three methods for extraction of volatile lipid oxidation products from food
 471 matrices for GC-MS analysis. *J. Am. Oil Chem. Soc* 2016, *93*, 929-942.

472 [18] Safafar, H., Hass, M. Z., Møller, P., Holdt, S. L. and Jacobsen, C. High-EPA biomass from
 473 nannochloropsis salina cultivated in a flat-panel photo-bioreactor on a process water-enriched
 474 growth medium. *Mar. Drugs* 2016, *14*, 1-19.

475 [19] Porter, N. A., Caldwell, S. E. and Mills, K. A. Mechanism of free radical oxidation of
 476 unsaturated fatty acids. *Lipids* 1995, *30*, 277-289.

477 [20] Frankel, E. N. Lipid oxidation: Mechanism, products and biological significance. *J. Am. Oil*
 478 *Chem. Soc* 1984, *61*, 1908-1916.

479 [21] van Ruth, M., Roozen, J. P. and Jansen, F. J. H. M. Aroma profiles of vegetable oils varying in
 480 fatty acid composition vs. concentrations of primary and secondary lipid oxidation products.
 481 *Nahrung* 2000, *44*, 318-322.

- 482 [22] Wright, R. H. and Serenius, R. S. E. Odour and molecular vibration. II.* Raman spectra of
483 substances with the nitrobenzene odour. *J. Appl. Chem.* 1954, 4, 615-621.
- 484 [23] Pollak, P., Romeder, G., Hagedorn, F. and Gelbke, H. Nitriles. *Ullmann's Encyclopedia of*
485 *Industrial Chem.* 2012, 24, 251-265.
- 486 [24] Khiari, D., Barrett, S., Chinn, R., Bruchet, A., Piriou, P., Matia, L., Ventura, F., Suffet, I.,
487 Gittelmann, T. and Leutweiler, P. Distribution generated taste-and-odor phenomena. *AWWARF* 2002,
488 Denver, USA.
- 489 [25] Lu, F. S. H., Nielsen, N. S., Baron, C. P. and Jacobsen, C. Oxidative degradation and non-
490 enzymatic browning due to interaction between oxidized lipids and primary amine groups in
491 different marine phospholipid emulsions. *Food Chem.* 2012, 135, 2887-2896.
- 492 [26] Jungermann, E. and Sonntag, N. O. V. Glycerine: a key cosmetic ingredients. *Marcel Dekker*
493 1991, New York, USA.
- 494 [27] Abe, H. and Bell, A. T. Synthesis of butyronitrile via ammonolysis of butylalcohol and
495 dehydrogenation of butylamine over Mo₂N. *J. Cat.* 1993, 142, 430-436.
- 496 [28] Bevington, J. C. The Decomposition of $\alpha\alpha'$ -azoisobutyronitrile in solution. *J. Chem. Soc.*,
497 1954, 1, 3707-3712.
- 498 [29] Krstina, J., Moad, G., Willing, R. I., Danek, S. K., Kelly, D. P., Jones, S. L. and Solomon, S. L.
499 Further studies on the thermal decomposition of AIBN-Implications concerning the mechanism of
500 termination in methacrylonitrile polymerization. *Eur. Polym. J.* 1993, 29, 379-388.
- 501 [30] Bennett, J. E., Brown, D. M. and Mile, B. Studies by electron spin resonance of the reactions
502 of alkylperoxy radicals. *J. Chem. Soc. Faraday* 1970, 66, 397-405.

Figure 1. Lipid oxidation and degradation products in PCF during 6 months storage at 5 °C (◆), 20 °C (●), 20 + light (▲), 40 °C (×) and 50 °C (●). The development of A) Peroxide value in meq/kg, B) Butanal, C) Butanenitrile and D) 1-Pentanol in ng/g.

Figure 1. Lipid oxidation and degradation products in PSF during 6 months storage at 5 °C (◆), 20 °C (■), 20 + light (▲), 40 °C (×) and 50 °C (●). The dotted line indicates the exact ODT value (determined in product) (butanal and pentanal). The development of A) Peroxide value in meq/kg, B) Butanal, C) 2-Pentanone, D) Pentanal, E) 2-Hexanone, F) Hexanal, G) 1-Octanol and H) Benzaldehyde in ng/g.

Figure 1. Volatile compounds [ng/g] present in skin conditioning and emollient raw materials during 3 months storage at 40 °C. A) tricaprilyn and tricaprins, B) glycerine, and C) coconut oil.

Figure 1. Volatile compounds [ng/g and response*10⁻³/g(only tetramethylbutanedinitrile)] present in texture modifying raw materials during 3 months storage at 40 °C. A) Lecithin and B) Polyacrylate crosspolymer-6.

Figure 1. AIBN decomposes to form 1. 2-cyanoprop-2-yl radicals, 2. Tetramethylbutanedinitrile, 3. Dimethyl-N-(2-cyanoprop-2-yl)ketenimine and 4. isobutanenitrile. A modification of Krstina *et al.* [29].

Table 1. The modifications for sample preparation, collection and water evaporation

Samples	Preparation	Collection	Evaporation
PSF	1 g sample. Incubation at 45 °C for 5 min.	50.0 mL/min at 45 for 10 min	50 ml/min at 25 °C for 22 min.
Tricaprylin and tricaprin Glycerine Coconut oil	1 g sample. Incubation at 60 °C for 4 min.	50.0 mL/min at 60 for 20 min	-
Lecithin Polyacrylate crosspolymer-6	1 g of sample and water were mixed(1:1). Incubation at 45 °C for 5 min.	50.0 mL/min at 45 for 10 min	50 ml/min at 25 °C for 22 min.

Table 2. Summary of volatile compounds present in both products and raw materials. + = present, ++ = present above ODT value in raw material (only available for butanal and pentanal), and - = absent.

Volatile compounds/ Raw material	Butanal	Pentanal	Hexanal	Benzaldehyde	Butanenitrile	1-Octanal	1-Pentanol	2-Pentanone	2-Hexanone
Tricaprylin and tricaprin	++	++	+	+	-	-	+	+	+
Glycerine	+	+	+	+	-	-	-	-	+
Coconut oil	-	++	+	+	-	-	-	-	+
Lecithin	+	++	+	+	-	-	-	+	+
Polyacrylate crosspolymer-6	++	++	+	+	-(?)	-	-	+	+