Investigation of Lipid Oxidation in the Raw Materials of a Topical Skin Formulation: A Topical Skin Formulation Containing a High Lipid Content

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

Published in:
Journal of the American Oil Chemists' Society

Link to article, DOI:
[10.1002/aocs.12015](https://doi.org/10.1002/aocs.12015)

Publication date:
2018

Document Version
Peer reviewed version

[Link back to DTU Orbit](#)

Citation (APA):
Thomsen, B. R., Taylor, R., Madsen, R., Hyldig, G., Blenkiron, P., & Jacobsen, C. (2018). Investigation of Lipid Oxidation in the Raw Materials of a Topical Skin Formulation: A Topical Skin Formulation Containing a High Lipid Content. *Journal of the American Oil Chemists' Society*, 95(2), 185-196. <https://doi.org/10.1002/aocs.12015>

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.

Investigation of lipid oxidation in the raw materials of a topical skin formulation: A topical skin formulation containing a high lipid content.

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

¹National Food Institute, Division of Food Technology, Technical University of Denmark, Lyngby, DK, ²Glaxo Smith Kline, Brentford, UK, ³Department of Chemistry, Technical University of Denmark, Lyngby, DK

Keywords

Oxidative Stability, Nutrition and Health, Autoxidation, Lipid Chemistry, Lipid Analysis and Lipids

Abstract

Several studies have demonstrated that lipid oxidation often occurs in topical skin formulations which can affect product odour (both positively and negatively). Furthermore, odour detection threshold values and odour descriptors of identified volatile oxidation products in cleansing and skin cream formulation prototypes were recently determined by a trained sensory panel at the Technical University of Denmark in the Division of Food Technology. In this study, we investigated lipid oxidation in a prototype skin cream formulation as well as in selected cosmetic skin care raw materials. Lipid oxidation was measured regularly over a six-month period for the product and over a three-month period for the raw materials by headspace gas chromatography–mass spectrometry. The volatile compound present in the highest initial concentration, and which increased most during storage, was 3-methyl-1-butanol (medicinal, chemical/cleaning agent odour), and its formation was linked to the raw material isoamyl p-methoxycinnamate. The odour character of the product after storage was assessed and informally deemed acceptable for consumer usage and typical of topical dermocosmetic products. A potential pathway for its formation was also identified. In addition, the concentrations of several well-known lipid oxidation products increased during storage and were suggested to originate primarily from rice bran wax, which oxidized more readily than other raw materials due to its unsaturated nature.

Introduction

Several studies have shown that lipid oxidation often occurs in topical skin care formulations containing unsaturated lipids and that lipid oxidation products can affect product quality (1–6) (i.e. odour (4–6) and colour (2)), potentially impacting product both positively and/or negatively.

1
2
3 30 Earlier studies have shown that raw materials were at least partly responsible for volatile compounds
4 31 present in simple emulsions immediately after their production (7,8). Since topical skin care
5 32 formulations are often emulsions, knowledge obtained from studies on simple emulsions can provide
6 33 some understanding of the mechanisms behind the formation of volatile compounds in topical
7 34 products. However, the composition of topical skin care formulations is far more complex than that of
8 35 simple emulsion systems and so are the oxidation mechanisms. In order to determine whether/which
9 36 raw materials are responsible for volatile compounds present in freshly produced topical skin
10 37 formulations, several factors must be considered: volatiles introduced by raw materials, production
11 38 method (e.g. temperature and other processing conditions as well as exposure to oxygen and light), and
12 39 the mechanisms leading to the formation of volatile compounds (7–12). Volatile lipid oxidation
13 40 products can also be formed during storage as a result of interactions between raw materials,
14 41 production method and storage conditions. Temperature and exposure to oxygen and light during
15 42 storage are factors that can influence the rate of lipid oxidation after production.
16
17
18
19
20
21
22
23
24
25 43 Other studies have investigated the effect of impurities in raw materials on oxidative stability in
26 44 finished food products and model emulsions, as summarised in a review by Waraho *et al.* (12), who
27 45 concluded that the oxidative stability of the finished product was linked to the quality of the raw
28 46 materials. Since some raw materials used in foods are common with cosmetics, studies performed on
29 47 raw materials for food can be used as guidance for cosmetics.
30
31
32
33
34
35 48 In a study on raw materials for personal care products, the impact of the production method on the
36 49 quality of myristyl myristate, a skin conditioning and opacifying agent, was explored (13). The purity
37 50 of myristyl myristate products varied from 80.1% to 97.5% between manufacturers. Furthermore, the
38 51 oxidative status of the myristyl myristate products measured by peroxide value (PV) fluctuated from
39 52 <0.1 to 6.0 meq/kg depending on the manufacturer and product grade. In addition, the colour, acid
40 53 value (0.2 - 0.8 mg/g), hydroxyl value (1.6 - 14.0 mg/g) and saponification value (128 – 134 mg/g)
41 54 also varied widely between the production methods used (13).
42
43
44
45
46
47 55 Two other studies investigated the oxidative stability of skin creams with new active ingredients, and
48 56 both studies showed significant changes in physical and oxidative stability as well as odour properties
49 57 as a result of the addition of extracts from Icelandic brown algae *Fucus vesiculosus* (2,14). This
50 58 highlights the importance of securing each raw material's quality, stability and an understanding of
51 59 raw material interactions.
52
53
54
55
56 60 The aim of this study was to explore lipid oxidation in selected raw materials and in a topical skin
57 61 formulation containing high levels of lipids. A second aim was to correlate any raw material oxidation
58
59
60

with the finished product oxidation to identify any culpable agents. In addition, we aimed to understand the mechanism leading to the formation of any identified volatile compounds.

Materials

Prototype Skin Cream Formulation (PSCF)

The prototype skin cream formulation was produced by GlaxoSmithKline (Brentford, United Kingdom) and contained several raw materials including rice bran wax, glycerine, isostearyl isostearate, palmitic acid monoethanolamine (PMEA). The prototype skin cream formulation contained approximately 29 % of lipid.

Raw materials

Separately to the aforementioned prototype product, individual (cosmetic-industry-relevant) raw materials were assessed for lipid oxidation potential:

- Rice bran wax (Koster Keunen, Bladel, Netherlands),
- Glycerine (Croda Europe Ltd, East Yorkshire, England),
- Isostearyl isostearate (Croda Europe Ltd, East Yorkshire, England),
- Palmitic Acid Monoethanolamine (PMEA; Jan Dekker, Wormerveer, Netherlands),
- Isoamyl p-methoxycinnamate (UV cinnamate) (Symrise AG, Holzminden, Germany),
- Bis-ethylhexyloxyphenol methoxyphenyl triazine (UV triazine) (BASF SE, Ludwigshafen, Germany),
- Hexyl 2-(1-(diethylaminohydroxyphenyl)methanoyl)benzoate (UV benzoate) (BASF SE, Ludwigshafen, Germany).

Methods

Storage conditions

PSCF was stored for 6 months at 5°C, 20°C and 40°C without exposure to light and at 20°C with exposure to light and for 2 weeks at 50°C. Samples were taken after 0, ½, 1, 2, 3 and 6 months.

Raw materials were stored at 40°C for 3 months; samples were taken after 0, 1, 2 and 3 months of storage.

88 The samples were stored in closed 40 ml opaque bottles. Samples were stored in individual bottles, to
89 be withdrawn at each time point for each analysis. After sampling, all samples were stored at 5°C until
90 analysis.

91 Oil extraction methodology

92 Oil was extracted from 5 g of PSCF and UV cinnamate with the Bligh and Dyer method (15) ($n = 2$).
93 However, a reduced amount of solvent was applied as described by Iverson *et al.* (16). In brief, lipids
94 were extracted by the use of a homogenous mixture of 20 ml of chloroform, 20 ml of methanol and 15
95 ml of water. The water soluble parts were separated from the lipid soluble parts by a subsequent
96 addition of chloroform and methanol. Phase separation was completed by centrifugation. After phase
97 separation was completed, chloroform in the chloroform and lipid phase was evaporated, and the oil
98 content could then be determined gravimetrically. The lipid extract was used as the starting material
99 for analysis of PV and determination of fatty acid composition.

100 Determination of Peroxide Value

101 PV was measured using the IDF method (17) and quantified by colorimetric determination of iron
102 thiocyanate spectrophotometrically at 500 nm by UV mini 1240 (Shimadzu, Duisburg, Germany) ($n =$
103 2). The spectrophotometer was reset to detect chloroform/methanol (7:3) solvent as zero.

104 Quantification of volatile compounds

105 Extraction of volatile compounds, GC-MS analyses and quantification were done automatically as
106 described by Thomsen *et al.* (18) with the following modification of the sample preparation, collection
107 and water evaporation (Table 1). These modifications were done in order to extract volatile
108 compounds from all matrices, to avoid contamination of the tube by powders and to remove water
109 residues.

110 Briefly, volatile compounds were collected from 1 g of sample in a 10 mL vial ($n = 3$). The automation
111 sequence was: incubation for 4 min at a temperature of 60 °C or 45 °C (see Table 1). The sample was
112 agitated at 300 rpm (agitator on time: 10 s, agitator off time: 1 s). Thereafter, purging with nitrogen at
113 50 ml/min through the headspace of the vial was started for 20 min. The volatile compounds were
114 trapped on tubes containing Tenax GR 300 (Gerstel GmbH & Co. KG., Mülheim an der Ruhr,
115 Germany). Water residues were removed from the tubes with a 50 mL/min purge flow (see Table 1).
116 Then the volatile compounds were desorbed from tubes in the thermal desorption unit (initial temp 40
117 °C, then 720 °C/min to 280 °C kept there for 5 min) to the GC. The volatile compounds were analysed

on a GC-MS model: HP 6890 - HP 5973 (Agilent Technologies, USA). Chromatographic separation was performed on a DB1701 column (30 m × ID 0.25 mm × 0.5 µm film thickness, J&W Scientific, Folsom, CA, USA) using helium gas flow (1.3 mL/min) in the GC. The MS settings were: 70 eV, electron ionization mode, mass to charge ratio (m/z) scan between 30 and 250. The GC temperature-program was as follows: initial 45°C, 5°C/min until 90°C, 4°C/min to 220°C and held for 4 min.

Fatty acid methyl esters (FAME)

Fatty acid compositions in oil and Bligh and Dyer extracts were determined as described by Safafar *et al.* (19) (n = 2). In brief, 1 g of Bligh and Dyer extract or 0.3 g of oil were weighed in test tubes. The chloroform was evaporated from Bligh and Dyer extract with nitrogen. Then, internal standard 23:0 was added to the oil and extracted together with heptane with BHT, toluene and borontrifluoride in methanol. Samples were mixed and methylated in a microwave oven (Microwave 3000 SOLV, Anton Paar, Ashland, VA, USA) and then cooled down. Saturated NaCl and heptane with BHT were added and thereafter phase separation occurred. The upper phase of the sample was transferred into 1 mL vials and analysed by Agilent 7890A gas chromatograph (Agilent Technologies, Palo Alto, CA, USA) with a DB-WAX fused silica capillary column (10 m×0.1 mm, 0.1 µm; Agilent Technologies, Palo Alto, CA, USA), helium as carrier gas and a flame ionization detector. The GC temperature program: initial 160 °C, 10.6 °C/min until 200°C 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 for 3.8 min. Fatty acids were identified by comparing their retention time to that of authentic standards. Fatty acids were expressed as % fatty acid of total fatty acids from C8-C24.

pH determination

The pH was measured using a Metrohm 827 pH meter (Metrohm, Herisau, Switzerland).

Description of difference scale

An expert panel of 3 scientists conducted a fast industry standard method to assess the odour changes. In this method, the sample odour was graded versus a reference sample stored at 5°C. The samples were ranked from one to five based on a scale description of difference (DOD) between sample and reference sample (Table 2). All samples ranked three or less were deemed within product range.

Statistical analysis

A two-way analysis of variance and a Bonferroni multiple comparison test were employed to evaluate significant changes in Figure 1 and 2. The significance level was 0.05. The statistical analysis was conducted using Graph Pad Prism version 6 (Graph Pad, La Jolla, USA).

Results and discussion

Lipid oxidation in PSCF: PV and volatile analysis

PV was used as a measurement of the primary oxidation products, lipid hydroperoxides. PV was initially 0.62 ± 0.01 meq/kg and remained below 0.65 meq/kg during the 6 months of storage at 5°C, 20°C and 40°C (data not shown). When exposed to light during storage, the PV increased slightly to 1.44 ± 0.17 meq/kg. According to PV, lipid oxidation only occurred to a low extent. However, a low PV does not necessarily imply that no oxidation has occurred; it may be related to rapid conversion of lipid hydroperoxides to secondary volatile oxidation products. It is therefore also advisable to assay for secondary lipid oxidation products.

The assay for secondary volatile oxidation products, via dynamic headspace GC-MS analysis, confirmed that the low PV was due to a fast conversion to aldehydes and alcohols. The concentration for the following volatile aldehydes increased significantly during storage (Figure 1): butanal, 3-methylbutanal, pentanal, hexanal, benzaldehyde and octanal. Butanal, pentanal, hexanal and octanal are all well-known lipid oxidation products. 3-methylbutanal and benzaldehyde have been suggested to originate from non-enzymatic browning reactions (20–22). Butanal, 3-methylbutanal, pentanal and hexanal increased to a greater extent during storage at 20°C and 40°C without exposure to light and at 20°C with exposure to light than at 5°C (Figure 1A-D). Unexpectedly, benzaldehyde and octanal increased most during storage at 20°C without exposure to light followed by 20°C with exposure to light.

In an earlier study, we determined odour detection threshold values for lipid oxidation products, which is the concentration at which the volatile compounds start to affect product odour. However, these were only determined for the volatile compounds that increased during storage in a PSCF. In general, we found that odour detection threshold values in PSCF were above 70 ng/g (5,6). Therefore, volatile compounds present in concentrations below 70 ng/g were not considered to affect product odour when present alone (3-methylbutanal and octanal) in the current study.

The odour detection threshold value determined for butanal was 72 ± 3 ng/g (5,6). In the present study, the concentration was above this level after 3 months storage at 20°C, 20°C with exposure to light or

40°C, and after 6 months at 5°C (Figure 1A). Butanal odour in PSCF has been described as parmesan and sour dishcloth (5,6).

The odour detection threshold value for pentanal (87 ± 5 ng/g) was slightly higher compared with butanal (5,6). The concentration was above this level after 3 months at 20°C with exposure to light (at 92 ng/g) or 40°C (at 104 ng/g), and after 6 months at 20°C or 5°C (Figure 1C). Pentanal odour in PSCF has been described as green and milk acidic (5,6). The odour detection threshold value for hexanal has not been determined in PSCFs. Based on the odour detection threshold values obtained for butanal and pentanal, it is estimated to be above 90 ng/g. Hexanal concentrations were above this level after 6 months of storage at all storage conditions. In literature, its odour has been described as fatty, green and fresh (23,24). In addition to aldehydes, a few alcohols and ketones increased as well (Figure 2).

The concentration of 3-methyl-1-butanol was significantly above its odour detection threshold value of 1926 ± 316 ng/g after 6 months of storage. Odour detection threshold values have not been determined for the ketones. However, none of the ketones increased to concentrations above 70 ng/g. Therefore, it is assumed that these ketones did not affect product odour. In a previous study, 3-methyl-1-butanol was described with the odour of glue, rubber, chemical, medicine, cleaning agent (5,6). An expert panel of 3 scientists conducted a DOD sensory evaluation to assess the odour changes, PSCF increased in intensity of chemical and cleaning agent, and scored 3 on the DOD scale after 6 months storage with exposure to light and at 40°C. Since many volatile compounds were present from the beginning of the storage period, they may originate directly from raw materials. Selected raw materials were explored to link volatile compounds in PSCF to those present in raw materials.

Lipid oxidation in selected raw materials

One of the primary functions of a cream is to moisturise and protect the skin so they often contain high levels of lipids, but unsaturated lipids can oxidize and form volatile compounds. Several volatile compounds were present initially in the lipid ingredients and more were generated during accelerated storage at 40°C in the following ingredients: rice bran wax and glycerine (Figure 3A and 3B). PSCF also contained D-panthenol, which was very stable during accelerated storage. Thus, benzaldehyde was the only volatile aldehyde that could be detected and this was not possible until 3 months of storage when 139 ± 9 ng/g was detected (data not shown).

Initially, some raw materials (rice bran wax and glycerine) contained several aldehydes and thus contributed to the initial concentration of all 10 volatile compounds detected in PSCF. Two raw

207 materials, rice bran wax and glycerine, contained butanal and contributed to the presence of this
208 volatile compound in the freshly produced PSCF. Furthermore, rice bran wax contained 1-pentanol at
209 262 ng/g and 2-pentanone at 6 ng/g after accelerated storage. Therefore, it is likely that these two raw
210 materials contributed to the development of 1-pentanol and 2-pentanone in PSCF. Moreover, the initial
211 content of pentanal, 3-methylbutanal, 2-hexanone and hexanal in PSCF originated partly from rice
212 bran wax and glycerine. The last aldehyde, benzaldehyde, may originate from D-panthenol (data not
213 shown), rice bran wax and glycerine.

214 Only low concentrations of volatile compounds were present in glycerine compared with rice bran
215 wax. Glycerine can oxidize to aldehydes such as glyceraldehyde in presence of metal ions and elevated
216 temperature. Overall, 11 different oxidation products that have a three carbon structure have been
217 identified for glycerine. However, the oxidation products can react with other molecules to form
218 compounds with more than three carbons. One proposed mechanism is a reaction between
219 glyceraldehyde and glycerine to form glycerine acetate described by Jungermann and Sonntag (25).
220 Another possibility is simple polymerisation. The purity of glycerine was 99.5%. Moreover, the
221 impurities may also contribute to the volatile compounds developing during accelerated storage.

222 Rice bran wax (mostly wax esters) mainly contained saturated fatty acids (86%; 16:0, 18:0, 20:0, 22:0
223 and 24:0), in addition to monounsaturated (6.5%; 18:1 n-9) and polyunsaturated fatty acids (3%; 16:3
224 n-4, 18:2 n-6, 20:3 n-6 and 20:4 n-6). Despite a low concentration of polyunsaturated fatty acids, rice
225 bran wax had significantly higher concentrations of most volatile compounds detected than glycerine
226 because polyunsaturated fatty acids were highly susceptible to auto-oxidation. Auto-oxidation of
227 polyunsaturated fatty acids gives rise to formation of primary oxidation product which can decompose
228 further to secondary oxidation products. One of most likely decomposition pathways is scission.
229 Scission (either α or β) results in a complex mixture of secondary oxidation products including the
230 measured alcohols, ketones and aldehydes (21,22).

231 The following two raw materials, PMEA and isostearyl isostearate, work as skin conditioners in PSCF.
232 Initially, only hexanal, butanal and pentanal were present in PMEA and isostearyl isostearate (Figure
233 4), and they may thus partly be responsible for the initial presence of hexanal in PSCF.

234 Several volatile compounds appeared in the raw materials during the 3 months of storage, but some of
235 these volatile compounds only appeared in PMEA and isostearyl isostearate (2-heptanone, heptanal
236 and nonanal) (Figure 4). However, all 10 volatile compounds that increased during storage in PSCF
237 also appeared and increased in isostearyl isostearate and PMEA, namely butanal, 3-methylbutanal
238 (only isostearyl isostearate), pentanal, 2-pentanone, 1-pentanol (only isostearyl isostearate), 3-methyl-

1
2
3 239 1-butanol (only isostearyl isostearate), hexanal, 2-hexanone, octanal and benzaldehyde (only PME).
4 240 Therefore, it is likely that PME and isostearyl isostearate contributed to the increase observed in
5
6 241 PSCF of most of the volatiles. The structure of both PME and isostearyl isostearate did not indicate a
7
8 242 clear reactive group/site, which can result in the observed volatile compounds. More studies are
9
10 243 therefore needed to understand where they originate from. Their presence may be related to impurities
11 244 present in the raw materials.

12
13
14 245 The last raw materials included in this study were UV filters. These raw materials were produced with
15 246 the purpose of being reactive towards pro-oxidants. Three UV filters were investigated: UV benzoate,
16 247 UV triazine and UV cinnamate. Initially, only a small amount of octanal was present in UV benzoate,
17 248 and UV triazine did not contain any known oxidation products (Figure 5).

18
19
20
21 249 After 3 months of accelerated storage, aldehydes predominantly formed in UV benzoate and UV
22 250 triazine. Some of the volatile compounds that appeared during the 3 months of storage were not
23 251 present in PSCF (heptanal and nonanal). UV benzoate and UV triazine generated butanal, 3-
24 252 methylbutanal (only UV benzoate), pentanal, hexanal, 2-hexanone, octanal and benzaldehyde after 3
25 253 months storage.

26
27
28
29
30 254 In contrast to the other two UV filters, UV cinnamate contained substantial amounts of 3-methyl-1-
31 255 butanol initially, and the concentration of this compound increased further during storage (Figure 5). In
32 256 addition, UV cinnamate also generated 3-methylbutanal during storage. After three months of
33 257 accelerated storage, octanal 28 ng/g, pentanal 42 ng/g and benzaldehyde 495 ng/g appeared as well
34 258 (Figure 5C). Although several aldehydes occurred after three months of storage, their concentrations
35 259 were low in UV filters compared with the concentrations in humectant, skin texture modifying and
36 260 skin conditioning raw materials. Therefore, UV benzoate and UV triazine were not explored further.
37 261 However, the high concentration of 3-methyl-1-butanol generated by UV cinnamate would be
38 262 expected to impact a finished product odour. A trained sensory panel described 3-methyl-1-butanol as
39 263 glue, rubber, chemical, medicine and cleaning agent (5,6). Therefore, it is important to understand the
40 264 route of reactions leading to 3-methyl-1-butanol in order to identify ways to control it.

41
42
43
44
45
46
47
48
49 265 MacManus-Spencer *et al.* (26) have previously investigated the degradation of octyl p-
50 266 methoxycinnamate under photolytic conditions and identified 4-methoxybenzaldehyde and 2-
51 267 ethylhexanol among the products. Two cleavage routes were considered in their work where the alkene
52 268 in the UV-filter either reacted with water followed by a retro-aldol reaction or a reaction occurred with
53 269 singlet oxygen to form the aldehydes through an unstable dioxetane (26). The same pathways can be
54 270 envisioned in our case where UV cinnamate either would form 4-methoxybenzaldehyde and isoamyl
55
56
57
58
59
60

acetate by reaction with water or undergo a cleavage with singlet oxygen to give the corresponding aldehydes (Scheme 1). The addition of water to cinnamates followed by a retro-aldol reaction is a known biosynthetic pathway in the synthesis of plant benzoic acids from cinnamates (27). As a result, it should also be a feasible chemical route although the transformation is probably very slow. The cleavage of olefins by singlet oxygen is well-known (28–30) and the formed isoamyl ester of glyoxylic acid is presumably labile enough to hydrolyse completely under the storage conditions (31). Finally, direct hydrolysis of UV cinnamate to the carboxylic acid and 3-methyl-1-butanol should also be included in the considerations (Scheme 1).

In addition to 3-methyl-1-butanol the degradation of UV cinnamate may thus also form 4-methoxybenzaldehyde and isoamyl acetate which can be used to distinguish between the different pathways. The pH of UV cinnamate was 4.23 initially and decreased slightly to 4.01 after 3 months storage at 40°C. Inspection of the chromatograms from UV cinnamate did indeed reveal the presence of both 4-methoxybenzaldehyde and isoamyl acetate. The retention time was 31.887 for 4-methoxybenzaldehyde and 14.024 for isoamyl acetate and both signals were confirmed by external standards. Notably, the acetate of the alcohol was not detected in the earlier work by MacManus-Spencer *et al.* (26). 4-Methoxybenzaldehyde and isoamyl acetate were both present in UV cinnamate from the beginning of the storage period and their amounts increased further during storage. Although, the two by-products have not been quantified by the use of calibration curves, they appear to be formed in somewhat equal amounts and certainly to a much lesser degree than 3-methyl-1-butanol, which is the main by-product. As a result, 4-methoxybenzaldehyde and 3-methyl-1-butanol cannot be formed by the oxidative cleavage with singlet oxygen since this would give rise to similar amounts of both compounds. Instead, it is very likely that 4-methoxybenzaldehyde and isoamyl acetate are formed by the addition of water and a retro-aldol reaction.

This leaves the direct hydrolysis of the ester as the main pathway for the formation of 3-methyl-1-butanol. It is known that esters can hydrolyse under near neutral conditions, but the reaction is very slow. For ethyl cinnamate the half-life for hydrolysis in water at pH 4.0 and 25 °C is estimated to be about 100 years (32). This number will be higher for UV cinnamate in the present case since the hydrolysis is slower in a non-polar environment. However, the amount of 3-methyl-1-butanol released in Figure 5 only corresponds to about 0.2‰ (w/w) after 3 months storage at 40°C. Therefore it is hypothesised that this is a result of a very slow direct hydrolysis of the ester in the UV-filter under the near neutral conditions.

Linking volatiles in PSCF with those in raw materials

The volatile compounds present in PSCF and raw materials are summarized in Table 3. In brief, the increase observed in butanal in PSCF during storage may mainly originate from isostearyl isostearate, for which the concentration was above odour detection threshold value from the beginning of the storage. However, butanal also developed in rice bran wax, glycerine, PMEA, UV triazine and UV benzoate during storage.

The formation of 3-methylbutanal was related to several raw materials, namely, rice bran wax, glycerine, isostearyl isostearate, UV cinnamate and UV benzoate. The concentration of pentanal increased in all raw materials and the concentration was above odour detection threshold value in rice bran wax, isostearyl isostearate and PMEA after 3 months of storage. Hexanal increased significantly in the PSCF during storage and also increased to high concentrations in rice bran wax and PMEA (more than 150 ng/g). In addition, hexanal was present in glycerine, isostearyl isostearate, UV triazine and UV benzoate in low concentrations (less than 70 ng/g). Benzaldehyde mainly increased in PSCF at 20°C with exposure to light to 112 ng/g after 6 months' storage. It was possible to relate benzaldehyde to all raw materials except isostearyl isostearate. The last aldehyde octanal appeared in all raw materials except glycerine during accelerated storage. Particularly the concentration of octanal increased in rice bran wax. The alcohol 1-pentanol marginally increased in a few materials, rice bran wax and isostearyl isostearate. In contrast, to the low concentration of 1-pentanol and 3-methyl-1-butanol in isostearyl isostearate, 3-methyl-1-butanol was present in high concentration in PSCF from the beginning and throughout the storage period. The RMs shown to generate 3-methyl-1-butanol during storage were UV cinnamate, glycerine and isostearyl isostearate. Lastly, the two ketones, 2-pentanone and 2-hexanone, were present in both PSCF and several raw materials but only in low concentrations.

GSK Toxicology group (2017) has assessed the human safety impact of the volatiles included in this report. At the determined levels these substances do not raise any toxicological concern, neither locally or systemically (33).

Conclusion

This study explored lipid oxidation and oxidative degradation in a topical skin formulation (PSCF) containing high levels of lipid. Some secondary volatile oxidation products were present initially and more were generated during the 6 months of storage. Most notably, 3-methyl-1-butanol was present in a high concentration initially and it increased further during storage. Since the concentration of 3-

1
2
3 334 methyl-1-butanol was higher than the odour detection threshold value after six months of storage, it
4 335 was expected to affect product odour after long term storage, generating an increase in the medicinal,
5 336 chemical/cleaning agent-type odour character. This product was therefore assessed for odour changes
6 337 (informally vs. a 5°C control sample) and deemed acceptable and typical of a dermocosmetic product,
7 338 highlighting again the importance of considering the combination effect (of other volatiles present) and
8 339 the product base odour when interpreting the impact of any lipid oxidation on product odour.
9
10
11
12
13 340 Selected raw materials were explored in order to link volatile compounds affecting the quality in the
14 341 topical skin formulation to raw material(s). The UV cinnamate filter developed high levels of 3-
15 342 methyl-1-butanol during storage so was identified as a material to control. A potential pathway leading
16 343 to 3-methyl-1-butanol was proposed.
17
18
19
20
21 344 Furthermore, well-known lipid oxidation products and non-enzymatic browning products were
22 345 suggested to originate from rice bran wax in particular because of its unsaturated nature. It was
23 346 surprising that volatile lipid oxidation products occurred in PMEA and isostearyl isostearate, as these
24 347 raw materials did not contain reactive sites for oxidation. More studies are needed to explore why
25 348 volatile compounds appeared.
26
27
28
29
30 349
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

References

1. Thanonkaew A, Wongyai S, Decker EA, McClements DJ (2015) Formation, antioxidant property and oxidative stability of cold pressed rice bran oil emulsion. *J Food Sci Technol* 52:6520–6528
2. Poyato C, Thomsen BR, Hermund DB, Ansorena D, Astiasarán I, Jónsdóttir R, Jacobsen C (2017) Antioxidant effect of water and acetone extracts of *Fucus vesiculosus* on oxidative stability of skin care emulsions. *Eur J Lipid Sci Technol* 119:1600072
3. Malinowska P, Gliszczynska-swiglo A, Szymusiak H (2014) Protective effect of commercial acerola, willow, and rose extracts against oxidation of cosmetic emulsions containing wheat germ oil. *Eur J Lipid Sci Technol* 116:1553–1562
4. Thomsen BR, Horn AF, Hyldig G, Taylor R, Blenkiron P, Jacobsen C (2017) Investigation of lipid oxidation in high- and low-lipid-containing topical skin formulations. *J Am Oil Chem Soc* accepted
5. Thomsen BR, Hyldig G, Taylor R, Blenkiron P, Jacobsen C (2017) Odour Detection Threshold Determination of Volatile Compounds in Topical Skin Formulations. *Eur J Lipid Sci Technol* submitted
6. Thomsen BR, Hyldig G, Taylor R, Gregory J, Blenkiron P, Jacobsen C (2016) Determination of threshold values of lipid oxidation products in skin care products. In: International Symposium on Lipid Oxidation and Antioxidants: PO-18. Porto, Portugal
7. Let MB, Jacobsen C, Meyer AS (2005) Sensory stability and oxidation of fish oil enriched milk is affected by milk storage temperature and oil quality. *Int Dairy J* 15:173–182
8. Let MB, Jacobsen C, Sørensen ADM, Meyer AS (2007) Homogenization conditions affect the oxidative stability of fish oil enriched milk emulsions: Lipid oxidation. *J Agric Food Chem* 55:1773–1780
9. McClements DJ (2005) Food Emulsions - Principles, Practices, and Techniques. London, UK: CRC Press:1-26, 167-525
10. McClements DJ, Decker EA (2000) Lipid Oxidation in Oil-in-Water Emulsions: Impact of Molecular Environment on Chemical Reactions in Heterogeneous Food Systems. *J Food Sci* 65:1270–1282

1
2
3 379 11. Schultz S, Wagner G, Urban K, Ulrich J (2004) High-pressure homogenization as a process for
4 380 emulsion formation. *Chem Eng Technol* 27:361–368
5
6
7 381 12. Waraho T, McClements DJ, Decker EA (2011) Mechanisms of lipid oxidation in food
8 382 dispersions. *Trends Food Sci Technol* 22:3–13
9
10
11 383 13. Heinrichs V, Thum O (2005) Biocatalysis for the production of care specialties. *Lipid Technol*
12 384 17:82–87
13
14
15 385 14. Garbossa WAC, Maia Campos PMBG (2016) Euterpe oleracea, Matricaria chamomilla, and
16 386 Camellia sinensis as promising ingredients for development of skin care formulations. *Ind*
17 387 *Crops Prod* 83:1–10
18
19
20
21 388 15. Bligh WJ, Dyer EGB (1959) A rapid method of total lipid extraction and purification. *Can J*
22 389 *Biochem Physiol* 37:911–917
23
24
25 390 16. Iverson SJ, Lang SL, Cooper MH (2001) Comparison of the Bligh and Dyer and Folch methods
26 391 for total lipid determination in a broad range of marine tissue. *Lipids* 36:1283–1287
27
28
29 392 17. Shantha NC, Decker EA (1994) Rapid, sensitive, iron-based spectrophotometric methods for
30 393 determination of peroxide values of food lipids. *AOAC Int* 77:421–427
31
32
33 394 18. Thomsen BR, Yesiltas B, Sørensen A-DM, Hermund DB, Glastrup J, Jacobsen C (2016)
34 395 Comparison of Three Methods for Extraction of Volatile Lipid Oxidation Products from Food
35 396 Matrices for GC–MS Analysis. *J Am Oil Chem Soc* 93:929–942
36
37
38
39 397 19. Safafar H, Hass MZ, Møller P, Holdt SL, Jacobsen C (2016) High-EPA biomass from
40 398 *Nannochloropsis salina* cultivated in a flat-panel photo-bioreactor on a process water-enriched
41 399 growth medium. *Mar Drugs* 14:144–153
42
43
44
45 400 20. Lu FSH, Nielsen NS, Baron CP, Jacobsen C (2012) Oxidative degradation and non-enzymatic
46 401 browning due to the interaction between oxidised lipids and primary amine groups in different
47 402 marine PL emulsions. *Food Chem* 135:2887–2896
48
49
50
51 403 21. Porter NA, Caldwell SE, Mills KA (1995) Mechanisms of free radical oxidation of unsaturated
52 404 lipids. *Lipids* 30:277–290
53
54
55 405 22. Frankel EN (1984) Lipid oxidation: Mechanisms, products and biological significance. *J Am*
56 406 *Oil Chem Soc* 61:1908–1917
57
58
59
60

- 1
2
3 407 23. Buttery RG, Turnbaugh JG, Ling LC (1988) Contribution of volatiles to rice aroma. *J Agric*
4 408 *Food Chem* 36:1006–1009
5
6
7 409 24. Venkateshwarlu G, Let MB, Meyer AS, Jacobsen C (2004) Chemical and Olfactometric
8 410 Characterization of Volatile Flavor Compounds in a Fish Oil Enriched Milk Emulsion. *J Agric*
9 411 *Food Chem* 52:311–317
10
11
12
13 412 25. Jungermann E, Sonntag NOV (1991) *Glycerine: A Key Cosmetic Ingredient*. New York, USA:
14 413 Marcel Dekker
15
16
17 414 26. MacManus-Spencer LA, Tse ML, Klein JL, Kracunas AE (2011) Aqueous photolysis of the
18 415 organic ultraviolet filter chemical octyl methoxycinnamate. *Environ Sci Technol* 45:3931–3937
19
20
21 416 27. Widhalm JR, Dudareva N (2015) A familiar ring to it: Biosynthesis of plant benzoic acids. *Mol*
22 417 *Plant* 8:83–97
23
24
25 418 28. Dellagrecia M, Iesce MR, Previtera L, Purcaro R, Rubino M, Zarrelli A (2008) Lignans by
26 419 photo-oxidation of propenyl phenols. *Photochem Photobiol Sci* 7:28–32
27
28
29 420 29. Murthy RS, Bio M, You Y (2009) Low energy light-triggered oxidative cleavage of olefins.
30 421 *Tetrahedron Lett* 50:1041–1044
31
32
33 422 30. Matsumoto, Masakatsu Kobayashi H, Matsubara J, Watanabe N, Yamashita S, Oguma D,
34 423 Kitano Y (1996) Effect of allylic oxygen on the reaction pathways of singlet oxygenation:
35 424 Selective formation of 1,2-dioxetanes from 1-alkoxymethyl-2-aryl-1-tert-butyl-2-
36 425 methoxyethylenes. *Tetrahedron Lett* 43:1319–1323
37
38
39
40
41 426 31. Brachais CH, Huguet J, Bunel C, Brachais L (1999) Identification of small molecules formed
42 427 from polymethyl glyoxylate degradation in vitro. *Polym Degrad Stab* 64:243–249
43
44
45 428 32. Rayne S, Forest K (2016) Carboxylic acid ester hydrolysis rate constants for food and beverage
46 429 aroma compounds. *Flavour Fragr J* 31:385–394
47
48
49 430 33. GSK (2017) Assessment of the human safety impact of the volatiles. GSK, Brentford, UK
50
51 431
52
53
54 432
55
56
57 433
58
59
60

Figure 1. Aldehydes increasing in PSCF during 6 months of storage at 5°C (◆), 20°C (□), 20°C with exposure to light (▲), 40°C (×) and 50°C (●). The dotted line indicates the odor detection threshold value (butanal and pentanal). The development of A) butanal, B) 3-methylbutanal, C) pentanal, D) hexanal, E) benzaldehyde and F) octanal during storage [ng/g]. Results are presented as average +/- SD and N=3. Significance differences at 0.05 level are only marked for the last sampling point.

Figure 1. Alcohols and ketones increasing in the PSCF during the 6 months of storage at 5°C (◆), 20°C (◐), 20°C with exposure to light (▲), 40°C (×) and 50°C (●). The dotted line is added for the exact threshold value (3-methyl-1-butanol). The development of A) 1-pentanol, B) 3-methyl-1-butanol, C) 2-pentanone, and D) 2-hexanone during storage [ng/g]. Results are presented as average $\pm$ SD and N=3. Significance differences at 0.05 level are only marked for the last sampling point.

Figure 3. Volatile compounds [ng/g] present in raw materials during the 3-month storage at 40°C. A) rice bran wax and B) glycerine. Results are presented as average +/- SD and N=3.

Figure 4. Volatile compounds [ng/g] present in skin texture modifying and skin conditioning raw materials during the 3-month storage at 40°C. A) PMEA and B) isostearyl isostearate. Results are presented as average +/- SD and N=3.

Figure 5. Volatile compounds [ng/g] present in UV filter raw materials during the 3 months of storage at 40°C. A) UV benzoate, B) UV triazine, and C) UV cinnamate. Results are presented as average +/- SD and N=3.

Scheme 1. Potential pathways for cleavage of UV cinnamate.

Table 1. Sample preparation, collection conditions and water evaporation applied for collection of volatile compounds from PSCF and raw materials.

Samples	Preparation	Collection	Evaporation
PSCF	1 g sample. Incubation at 45°C for 5 min.	50 mL/min at 45°C for 10 min	50 ml/min at 25°C for 22 min.
Rice bran wax Glycerine Isostearyl isostearate UV cinnamate	1 g sample. Incubation at 60°C for 4 min.	50 mL/min at 60°C for 20 min	-
PMEA UV triazine UV benzoate	1 g of sample and water were mixed (1:1). Incubation at 45°C for 5 min.	50 mL/min at 45°C for 10 min	50 ml/min at 25°C for 22 min.

Table 2. Description of difference scale.

<u>DOD Scale</u>	<u>Description of Difference</u>
1	No differences in character or intensity noted
2	Reasonably sure difference exists, though difference may be too subtle to accurately describe
3	Definite difference, can describe difference with reasonable surety
4	Product or material out of expected range. Moderate or large intensity differences or ANY character differences.
5	Outside normal range. Large intensity and/or character differences.

Note: DOD = Degree of Difference

Table 3. Summary of volatile compounds present in both PSCF and raw materials. + = present, ++ = present above threshold value (5,6) in raw material (only available for butanal, pentanal and 3-methyl-1-butanol), and - = absent.

Volatile compounds/ Raw material	Butanal	3-methylbutanal	Pentanal	Hexanal	Benzaldehyde	Octanal	1-pentanol	3-methyl-1-butanol	2-pentanone	2-hexanone
Rice bran wax	+	+	++	+	+	+	+	-	+	+
Glycerine	+	+	+	+	+	-	-	+	-	+
Isostearyl isostearate	++	+	++	+	-	+	+	+	+	+
PMEA	+	-	++	+	+	+	-	-	+	+
UV cinnamate	-	+	+	-	+	+	-	++	-	-
UV triazine	+	-	+	+	+	+	-	-	-	+
UV benzoate	+	+	+	+	+	+	-	-	-	+