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1 **Improving oxidative stability of skin care emulsions with antioxidant extracts**
2 **from brown alga *Fucus vesiculosus***

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9

10 **Abstract**

11 Skin care products are known delivery systems of functional lipids, which can enhance the natural
12 defense of the skin. Unsaturated lipids, e.g. linoleic acids, are more susceptible to lipid oxidation
13 than saturated lipids. Therefore, lipid oxidation must be prevented to preserve the functionality of
14 the unsaturated lipids in skin care products.

15 The antioxidant properties of two *Fucus vesiculosus* extracts (water (WE) and 80% (v/v) ethanol
16 extracts (EE)) were evaluated. Both extracts had high in vitro antioxidant properties and high
17 phenolic content. The antioxidant efficacy of the extracts was evaluated by addition of 0.05 and 0.1
18 % (w/w) freeze dried extracts to facial cream formulations. The cream was stored up to 42 days
19 (dark, 20°C) and the oxidative stability was determined by following tocopherol consumption and
20 development in peroxides and secondary oxidation products. The results showed that EE was able
21 to reduce the peroxide oxidation rate from 84.8% (control without extract) to 41.3%. Furthermore, a
22 higher efficacy was observed for EE over WE. This was most likely related to higher phenolic
23 content, high radical scavenging activity and moderate metal chelating ability. However, WE
24 indicated regeneration of tocopherols by amphiphilic polyphenolic phlorotannins, with interfacial

25 properties. The results show that *F. vesiculosus* extracts rich in antioxidative phlorotannins, can
26 play an important role in reducing lipid oxidation and maintaining quality of cream.

27

28 Key words: Emulsion, lipid oxidation, quality, antioxidant, seaweed, phlorotannin

29

30 **1. Introduction**

31 Skin care products such as facial cream typically contain 12-22% (w/w) oils, e.g. almond or apricot
32 oil. Almond oil is rich in unsaturated fatty acids, such as oleic and linoleic acid, which provide
33 different functional properties to the product such as skin hydration and skin strengthening.

34 However, these unsaturated fatty acids can undergo lipid oxidation, leading to loss of their
35 functionality, quality deterioration of the product (rancidity) and formation of reactive oxygen
36 species (ROS). ROS can lead to oxidative stress and inflammatory conditions of the skin and can
37 furthermore result in premature skin aging (Kozina, Borzova, Arutiunov, & Ryzhak, 2013). To
38 control lipid oxidation, antioxidants can be added. Due to increasing demands for clean labeling and
39 natural ingredients, natural additives are of interest to the cosmetic industry.

40 In recent years, brown algae have aroused the interest of many researchers due to the presence of a
41 variety of bioactive compounds and their nutritional value. A number of potent antioxidant
42 compounds have been isolated and identified from different types of brown algal species, including
43 polyphenols (phlorotannins), polysaccharides (fucoidans and laminarin), carotenoids (fucoxanthin
44 and astaxanthin), monophenols and catechins (Hold & Kraan, 2011).

45 A number of studies have reported that seaweed extracts demonstrated strong antioxidant properties
46 due to the presence of polyphenolic secondary metabolites, phlorotannins, which is the dominant
47 polyphenolic group in brown algal (Chkhikvishvili & Ramazanov, 2000; Wang, Jónsdóttir &
48 Ólafsdóttir, 2009; Farvin & Jacobsen, 2013). Farvin and Jacobsen (2013) screened 8 different

49 seaweed species collected along the Danish coast, and found that among the brown alga, *Fucus* sp.
50 (*Fucus vesiculosus* and *Fucus serratus*) showed higher radical scavenging activity and polyphenol
51 content compared to other species such as *Laminaria*. Moreover, they found that the total phenolic
52 content (TPC) increased significantly when the polarity of the extraction solvent decreased (TPC:
53 water < ethanol), indicating that the extraction yield of phlorotannins can be increased by using
54 aqueous solutions of ethanol (up to 80%).

55 Previously, both water and ethanolic extracts from Icelandic *F. vesiculosus* were able to prevent
56 lipid oxidation in different food systems and cosmetic emulsions due to a high content of
57 phlorotannins found in the seaweed species (Hermund, Yesiltas, Honold, Jónsdóttir, Kristinsson,
58 Jacobsen, 2015; Honold, Jacobsen, Jónsdóttir, Kristinsson & Hermund, 2016; Karadag, Hermund,
59 Jensen, Andersen, Jónsdóttir, Kristinsson & Jacobsen, 2017; Poyato, Thomsen, Hermund,
60 Ansorena, Astiasarán, Jónsdóttir, Kristinsson & Jacobsen, 2017). In these studies it was concluded
61 that the antioxidant efficacy of the *F. vesiculosus* extract is highly dependent on the *in vitro*
62 antioxidant properties, since different antioxidant properties are required to protect complex food
63 systems or cosmetic emulsions. The exact composition of the food system or cosmetic emulsion
64 will determine which antioxidant properties are the most important in order to obtain high efficacy.
65 Hence, there is a need to further study the antioxidant efficacy of extracts from *F. vesiculosus* in
66 preventing lipid oxidation in skin care emulsions such as facial cream. Other functional properties
67 of brown alga extracts rich in phlorotannins such as antimicrobial and anti-aging activity have been
68 reported (Sugiura, Matsuda, Yamada, Imai, Kakinuma, & Amano, 2008; Lee, Kang, Hwang, Eom,
69 Yang, Lee, Lee, Jeon, Choi, & Kim, 2008). Therefore, there is a great potential to develop natural
70 multi-functional ingredients from seaweed to support the natural defense of the skin.

71 The objectives of the present study were to evaluate the antioxidant potential of two different
72 extracts (a water extract and an 80% (v/v) ethanol extract) from Danish *F. vesiculosus* to increase

73 the oxidative stability of facial cream and to protect functional lipids. The antioxidant composition
74 of the extracts was determined, and their antioxidant properties studied using three *in vitro* assays;
75 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity, reducing power, and metal
76 chelating activity.

77 In a previous study by Poyato et al. (2017) a similar experiment was carried out for *F. vesiculosus*
78 extracts (acetone and water) added to facial cream in concentrations of 1 and 2 g/kg cream (0.1 and
79 0.2%). In the present study, we wanted to evaluate lower concentrations of water extract and also
80 include an ethanol extract since previous studies have shown very similar antioxidant activity, both
81 *in vitro* and in food systems, and chemical composition of ethanol and acetone extracts.

82

83 **2. Materials and method**

84 **2.1. Alga material**

85 *Fucus vesiculosus* was collected by hand in September 2016 from the intertidal zone of Bellevue
86 beach (55°46'17.4"N 12°35'48.4"E), north of Copenhagen, Denmark. The seaweed was rinsed with
87 distilled water and their holdfasts and epiphytes were removed. Thereafter, the rinsed seaweed was
88 frozen in sealed plastic bags (-40°C) until further use. The seaweed samples were freeze-dried for
89 72h and milled (using a kitchen blender) into a fine powder. The powdered seaweeds were stored at
90 -80°C in sealed plastic bottles until extraction.

91

92 **2.2. Raw materials and solvents**

93 The raw materials used to produce the facial cream were purchased from Urtegaarden (Allingåbro,
94 Denmark): Aloe vera water, glycerine, sodium stearoyl lactylate, glyceryl palmitate, sodium
95 benzoate, almond oil, lanette wax, and vitamin E. The almond oil had a peroxide value (PV) of 0.81
96 meq O₂/kg oil, a free fatty acid content of 0.1±0.0 %, and the fatty acid composition in % of total

97 fatty acids (> 0.5%) of the almond oil were as follows: 16:0 (5.5%), 16:1 (0.7%), 18:0 (1.9%), 18:1
98 (n-9) (62.0%), 18:1 (n-7) (1.7%), 18:2 (n-6) (20.5%), 18:2 (n-4) (0.8%), 20:0 (0.6%), and 20:1 (n-7)
99 (2.9%). The vitamin E had a tocopherol content of: 62.7 mg α -toc/g oil, 8.6 mg β -toc/g oil, 362.2
100 mg γ -toc/g oil and 103.9 mg δ -toc/g oil. The solvents used were of HPLC grade and purchased from
101 Lab-Scan (Dublin, Ireland). Standards and reagents were purchased from Sigma-Aldrich
102 (Steinheim, Germany).

103

104 **2.3. Extraction of antioxidants**

105 Extraction was performed according to Wang et al. (2009) using traditional solid-liquid extraction
106 (SLE) method. Water or 80% (v/v) ethanol were used as extraction solvents. For the preparation of
107 extract, 5 g of powdered seaweed were added to 100 mL water or 80% (v/v) ethanol and shaken
108 vigorously for 30 sec. Extraction was carried out for 24h in the dark at 20°C using a platform shaker
109 (Heidolph Instruments, Unimax 2010, Schwabach, Germany) at 125rpm. Afterwards, the extracts
110 were centrifuged at 1665g for 10 min. The supernatant was collected after passing through a filter
111 paper (Whatman 4, 20-25 μ m) and the residue was re-extracted once under the same conditions as
112 mentioned above and the supernatants were pooled (total of 200 mL extract solution). Hence, two
113 extracts were obtained, water extract (WE) and 80% (v/v) ethanol extract (EE).

114 The extraction procedure was repeated to evaluate the reproducibility of the SLE method applied.

115 The extraction reproducibility was evaluated by total phenolic content and no significant ($p < 0.05$)
116 difference between the two replicates was found (data not shown). For the antioxidant properties
117 (radical scavenging capacity, metal chelating ability and reducing power) of the extract solutions
118 were determined by EC50 or EC0.5 values (μ L extract solution/mL total volume). For the chemical
119 analysis the extracts were freeze dried and pooled. Furthermore, a storage trial to evaluate the

120 antioxidant efficacy and stability in facial cream was carried out using the pooled freeze dried
121 extracts. Both extract solutions and freeze dried extract were stored at -80°C until use.

122

123 **2.4. Extract characterization and antioxidant evaluation**

124 ***In vitro* antioxidant properties.** The extract solutions were diluted to different concentrations (0-
125 0.75 mL extract solution in 1 mL total volume) with water for determination of the EC50 values
126 (the concentration where 50 % inhibition was obtained in the different antioxidant assay) in a
127 dose/response curve (section 2.6.3). Three spectrophotometric assays were applied: DPPH (1,1-
128 diphenyl-2-picrylhydrazyl) radical scavenging, iron chelating and reducing power assay. For
129 reducing power the effective concentration to obtain an absorbance of 0.5 (EC50) was determined.
130 In all cases, a sample blank was included to eliminate the influence of colour from the extracts. In
131 all assays were performed in triplicates.

132 ***DPPH radical scavenging capacity.*** The assay was based on the method by Yang, Guo & Yuan
133 (2008) and modified for use in 96-well microtiter plates. In brief, 100 µL sample was loaded to the
134 microtiterplate and mixed with 100 µL 0.1 mM DPPH (in ethanol). After 30 min incubation (room
135 temperature, dark) the absorbance was measured at 517 nm. BHT was used as a positive control (10
136 mg in 50 mL ethanol exhibit approximately 70% inhibition in the assay).

137 ***Metal chelating ability.*** This assay was performed according to Farvin, Baron, Nielsen & Jacobsen
138 (2010). In short, 100 µL sample and 110 µL water was mixed in the microtiter plate. A solution of
139 20 µL 0.5 mM ferrous chloride was added to the solution and incubated for 3 min before 20 µL 2.5
140 mM ferrozine was added. After 10 min of incubation (room temperature, dark) the absorbance was
141 measured at 562 nm. EDTA (0.5 M) was used as a positive control (approximately 99% inhibition
142 in the assay).

143 *Reducing power.* This assay was modified from Yang et al. (2008). With the modifications the
144 assay description was as follows: A mixture of 200 μ L sample, 200 μ L 0.2 M phosphate buffer (pH
145 6.6) and 200 μ L 1% potassium ferricyanide was incubated in a water bath at 50°C for 20 min.
146 Afterwards, 200 μ L 10% TCA (trichloroacetic acid) was added to stop the reaction. 100 μ L reaction
147 solution was loaded to a microtiter plate and mixed with 100 μ L water. Then, 20 μ L 0.1% ferric
148 chloride was added and the mixture was incubated for 10 min (room temperature, dark). The
149 absorbance was measured at 700 nm. Ascorbic acid (0.5 mM) was used as a positive control and
150 gives approximately an OD700 of 0.8 in the assay.

151

152 **Total phenolic content (Folin–Ciocalteu).** TPC was determined with Folin–Ciocalteu assay and
153 used as an estimate for the phlorotannin content, the major polyphenolic group in *F. vesiculosus*
154 (Wang et al., 2009; Farvin & Jacobsen, 2013; Hermund et al., 2015). The quantification was carried
155 out according to Farvin and Jacobsen (2013). In brief, 100 μ L extract solution (2 mg dry weight/mL
156 methanol) was mixed with 0.75 mL of Folin–Ciocalteu reagent (10 % w/w in distilled water). After
157 5 min, 0.75 mL of sodium carbonate (7.5 % w/w in distilled water) was added. The samples were
158 incubated for 1.5 h at room temperature in the dark. Then, 200 μ L were transferred to a microtiter
159 plate and the absorbance measured at 725 nm with an UV–Vis spectrophotometer (Shimadzu UV
160 mini 1240, Duisburg, Germany). A standard curve from gallic acid (GA) was made for calibration
161 (concentrations from 0 to 500 μ g/mL). The analysis was performed in triplicates. The results are
162 expressed as GA equivalent (GAE) g/100 g dry weight.

163

164 **Pigments.** Prior to analysis, the freeze dried extract powder was dissolved in methanol (1-2 mg/mL
165 methanol) and filtered with syringe filter (0.22 μ m). The analysis of pigments was based on an
166 HPLC method of Van Heukelem and Thomas (2001) with some modifications described by Honold

167 et al. (2016). Pigment analysis was performed on an Agilent 1100 Series HPLC (Agilent
168 Technology, CA, USA) equipped with a diode array detector (DAD). Separation was obtained on a
169 ZORBAX Eclipse XDB-C8 column (150x4.6mm) with a particle size of 3.5µm (Agilent, CA, USA)
170 and a solvent gradient consisting of A: 70% methanol with 30% 0.028 M tetra butyl ammonium
171 acetate in water (pH = 6.5), and B: absolute methanol. The programme started at 5 % B increasing
172 to 95 % B in 27 min and held for 7 min at 95 %, further increased to 100% B in 1 min and held for
173 3 min, before decreasing from 100 to 5% B in 2 min, and held for 6 min. Injection volume was 100
174 µL, and the flow rate was 1.1 mL/min. The oven temperature was set to 60 °C. Calibration was
175 performed using external standards (chlorophyll c3, chlorophyll c2, peridin, fucoxanthin,
176 neoxanthin, prasinaxanthin, violaxanthin, diadinoxanthin, alloxanthin, zeaxanthin, leutin,
177 cataxanthin, chlorophyll B, chlorophyll, α - and β -carotene). 19-but-Fucoxanthin and chlorophyllide
178 were quantified as equivalent to fucoxanthin and chlorophyll, respectively. Pigments were detected
179 by fluorescence at 450 and 440 nm. For the internal standard (tocopherol acetate), the wavelength
180 was 222 nm. The analysis was performed in duplicate, and the results are expressed in µg/g freeze
181 dried extract.

182

183 **Phenolic compounds.** Prior to analysis, the freeze dried extract was dissolved in methanol (1-2
184 mg/mL methanol) and filtered with syringe filter (0.22 µm). The phenolic compounds were
185 analysed based on the method described by Farvin and Jacobsen (2013) using HPLC. The phenolic
186 extracts were analysed on an Agilent 1100 Series HPLC (Agilent Technology, CA, USA) equipped
187 with a DAD. The phenolic compounds were separated using Prodegy 5u ODS (250x4.6mm) with a
188 particle size of 5 µm (Phenomenex) with a guard and A: water with phosphoric acid (pH 3) and B:
189 methanol:acetonitrile (50:50). The gradient was as follows: 0-99% B in 40 min and held for 5 min.
190 Injection volume was 20µL and the flowrate was 0.9 mL/min. The analysis was performed at room

191 temperature. The detection was obtained at a wavelength of 210, 235, 255 and 280 nm. The
192 quantification was done using calibration curves with external standards (phloroglucinol, gallic
193 acid, 4-hydroxybenzoic acid, syringic acid, p-coumaric acid, quercetin, naringenin) in the
194 concentration range from 0-0.18 mg/mL methanol. Analysis was performed in triplicates. Results
195 are expressed in mg/g freeze dried extract.

196

197 **2.5. Facial cream production**

198 Facial cream was produced according to the recipe found in Poyato et al. (2017) and the water (WE)
199 and ethanol extract (EE) were added. Five codes were produced (WEC1, WEC2, EEC1, EEC2 and
200 CON) using the following amounts of ingredients (w/w%): Water phase contained: 53% water, 10%
201 Aleo vera water, 6.3% glycerine, 3.6% MF fat (water phase emulsifier), 0.6% natriumbenzoate. Oil
202 phase contained: 21.8% almond oil, 2% lanette, and 1.6 VE fat (fat phase emulsifier), 0.9% vitamin
203 E. The water phase and the oil phase were heated separately to 75°C to melt the ingredients
204 together. Directly after heating, the extracts were added to the water phase in two concentrations,
205 (0.05 and 0.1g dw/100 g skin care emulsion) and the oil phase was poured slowly into the water
206 phase under powerful steering (Ultra Turrax® T25Basic, 9500 rpm, IKA, NC, USA) for 5 min,
207 until the emulsion started to thicken. The emulsion was cooled down at room temperature before it
208 was dispersed in 50 mL clear PP containers (30 g in each) and sealed with a PP lid. Cream was
209 stored at room temperature in the dark for up to 6 weeks (42 days).

210

211 **2.6. Determining oxidative and physical stability of facial cream**

212 **2.6.1. Oxidative stability**

213 **Lipid extraction.** Lipids were extracted from the facial cream prior to analysis according to the
214 method described by Iverson, Lang & Cooper (2001) based on the method of Bligh & Dyer (1959).

215 Five grams of cream were used for the oil extraction. For each sample, two oil extractions were
216 performed and analyzed independently. Lipid extracts were subsequently used for the analysis of
217 peroxides, fatty acid composition and tocopherol content.

218

219 **Fatty acid composition.** The fatty acid composition of the oil phases was determined after fatty
220 acid methylation and analysis by GC paired with flame ionization detection (GC-FID) according to
221 Poyato et al. (2017). The FAME analysis was carried out using GC (HP 5890A, Agilent
222 Technologies, Palo Alto, CA, USA) according to AOCS (1998). For separation DB127-7012
223 column (10 m x ID 0.1 mm x 0.1 μ m film thickness, Agilent Technologies, Palo Alto, CA, USA)
224 was used. Injection volume was 0.2 μ L in split mode (1:50). The initial temperature of the GC-oven
225 was 160°C. The temperature was gradually increased as follows: 160-200°C (10.6°C/min), 200°C
226 kept for 0.3 min, 200-220°C (10.6°C/min), 220°C kept for 1 min, 220-240°C (10.6°C/min) and
227 kept at 240°C for 3.8 min. The measurements were performed on samples from storage day 0, 21
228 and 42, in duplicates, and the results were given as peak area in % of total area.

229

230 **Tocopherol content.** Two grams of lipid extracts from the facial cream were evaporated under N₂
231 to remove chloroform, and dissolved in 1.0 mL heptane for this analysis. The samples were
232 analyzed by HPLC (Agilent 1100 Series, Agilent Technology) according to AOCS (2009) to
233 quantify the contents of α -, β -, γ - and δ -tocopherols. 40 μ L were injected and the tocopherol
234 homologues were separated using a silica column (Waters, Dublin, Ireland, 150 mm, 4.6 mm, 3 μ m
235 silica film) and detected by UV-vis (292, 296 and 298 nm). Elution was performed isocratically
236 with 75:8:17 (ACN/MeOH/water, v/v/v) containing 0.2% acetic acid. A stock solution containing
237 10 mg tocopherols (mixture of α -, β -, γ - and δ -tocopherols) per liter was prepared to determine the
238 retention time of the tocopherols and the peak areas of the standards in order to calculate the

239 tocopherol content of the samples. The analyses were performed in duplicate at all sampling points
240 (day 0, 3, 7, 14, 21, 28 and 42), and results are reported as μg α -, β -, γ - or δ -tocopherols/g cream.

241

242 **Peroxide value (PV).** PV was determined according to the method by Shantha & Decker (1994),
243 based on the formation of an iron-thiocyanate complex. The colored complex was measured
244 spectrophotometrically at 500 nm (Shimadzu UV1800, Shimadzu Scientific Instruments, Columbia,
245 MD, USA). The analyses were done in duplicate at all sampling points (day 0, 3, 7, 14, 21, 28 and
246 42), and the results were expressed in milliequivalents peroxides per kg oil (meq O_2 /kg oil).

247

248 **Volatile compounds.** The secondary volatile oxidation products were collected by dynamic
249 headspace (DHS) and analyzed by GC-MS. This method is a valid and recognized qualitative
250 method for determining secondary oxidation products in oil and emulsions (Hartvigsen, Lund,
251 Hansen & Holmer, 2000).

252 Tenax GRTM packed tubes were used to collect volatile compounds. The collection was carried out
253 using 4 g of emulsion including 30 mg internal standard (4-methyl-1-pentanol) and 20 mL of water.
254 The volatile secondary oxidation products were collected at 45 °C under purging with nitrogen
255 (flowrate of 150 mL/min) for 30 min under constant shaking to avoid foam formation, followed by
256 flushing the Tenax GRTM packed tube with nitrogen (flow of 50 mL/min for 5 min) to remove
257 water. The trapped volatiles were desorbed using an automatic thermal desorber (ATD-400, Perkin-
258 Elmer, Norwalk, CT) connected to an Agilent 5890 IIA model gas chromatograph equipped with a
259 HP 5972 mass selective detector. The settings for the MS were: electron ionization mode, 70 eV,
260 mass to charge ratio (m/z) scan between 30 and 250 mAU. Chromatographic separation of volatile
261 compounds was performed on a DB1701 column (30m \times ID 0.25mm \times 0.5 μm film thickness, J&W
262 Scientific, Folsom, CA, USA) using helium gas flow (1.3 mL/min).

263 The temperature programme was as follows: 3 min at 35°C, 3°C/min from 35 to 120°C, 7°C/min to
264 120-160°C, 15°C/min 160-200°C and hold for 4 min at 200°C.

265 The auto sampler collector setting details were: 9.2 psi, outlet split: 5.0 mL/min, desorption flow:
266 60 mL/min. The analysis was performed in triplicate at all sampling points and the results were
267 given in ng/g cream.

268 A standard solution (standards in methanol) from which a dilution row were prepared and 1 µL of
269 each concentration prepared were added to a Tenax GR™ tube and flushed with nitrogen (flow of
270 50 mL/min for 5 min) to remove the solvent. Then, the volatiles were analyzed in the same way as
271 for the samples and calibration curves were calculated and used for calculation of concentrations of
272 volatiles in the samples (ng/g product).

273 Degree of difference (DOD) testing was also performed in order to evaluate the off odor of the
274 creams added seaweed extracts compared to the control. Hence, this preliminary testing was
275 performed by two personnel expert panel at day 0, 21 and 42. A 1 to 5 degree of difference scale
276 was used (Aust, Garcula Jr., Beard & Washam II, 1985), where 1 was “no difference in character or
277 intensity” and 5 was “Outside normal range. Large intensity and/or character difference”. The
278 results are discussed in relation to the results of secondary oxidation products.

279

280 **2.6.2. Physical stability**

281 **Droplet size distribution.** The size of fat droplets in the o/w emulsion systems was determined by
282 laser diffraction using a Mastersizer 2000 (Malvern Ins., Worcestershire, UK). The cream was
283 diluted 1:9 in SDS buffer (10 mM NaH₂PO₄, 5 mM SDS, pH 7) and sonicated in a water bath for 15
284 min at room temperature prior to analysis. Drops of diluted cream were added to recirculation water
285 (3000 rpm) reaching an obscuration of 14-17%. The refractive index (RI) of sunflower oil at 1.469
286 and water at 1.330 were used as particle and dispersant, respectively. Measurements were

287 performed in triplicates on days 0 and 42. Results were given as surface area mean diameter $D[3,2]$
288 $= \Sigma d^3/d^2$ (Rawle, 1996).

289

290 **Color determination.** Color of the facial cream emulsions were determined by a digital colorimeter
291 (Chromameter-2 CR-200, Minolta, Osaka, Japan). It measures three parameters, L^* , a^* and b^* . L is
292 lightness which ranges from white to black; a^* is redness which ranges from red (positive value) to
293 green (negative value) and b^* is yellowness which ranges from yellow (positive value) to blue
294 (negative value). Measurements were performed in triplicates on days 0, 21 and 42.

295

296 **2.6.3. Data treatment**

297 In order to determine the EC50 values (efficient concentration at 50% inhibition) of the extracts in
298 the antioxidant *in vitro* assays, dose/response curves were made. Linear regression was performed
299 on the linear part of the exponential phase and based on the linear function EC50 were determined
300 for DPPH radical scavenging capacity, iron chelating ability and the reducing power, respectively,
301 for each extract. These values and standard deviations (\pm SD) were calculated based on triplicates.
302 For all results mean and standard deviation were calculated and the results were analyzed by two-
303 way ANOVA (GraphPad Prism Version 7.0, GraphPad Software, Inc.). The Bonferroni multiple
304 comparison post-test was used to test difference between samples or storage time. The results are
305 considered to be significant when p -value ≤ 0.05 .

306

307 **3. Results**

308 **3.3. Extract characterization**

309 The aim was to apply solid-liquid extraction (SLE) using water and aqueous ethanol solutions to
310 obtain a high yield of the phlorotannins. Folin ciocaltau assay was used to estimate the total

311 phenolic content (TPC) as an indication of the phlorotannin content in the extracts. However, more
312 sensitive analytical approaches should be used for identification of specific phlorotannins as
313 suggested by Hermund, Plaza, Turner, Jónsdóttir, Kristinsson, Jacobsen & Nielsen (2018).
314 Both water and the aqueous solution with 80% (v/v) ethanol successfully extracted phenolic
315 compounds according to the estimation of the total phenolic content (TPC) shown in Table 1. The
316 use of ethanol increased the polyphenolic yield significantly from ca. 13.5 to ca. 16.5 g GAE/100g
317 dry weight ($p < 0.05$). These results are in agreement with previous studies that found that the
318 phenolic yield from *F. vesiculosus* decreased with decreasing polarity of the extraction media
319 (Farvin & Jacobsen, 2013; Hermund et al., 2015; Honold et al., 2016).
320 Hermund et al. (2015) and Honold et al. (2016) found higher TPC in their water and 80% (v/v)
321 ethanolic extracts derived from Icelandic *F. vesiculosus* compared to the Danish species in the
322 present study, 18.4±0.1 g GAE/100g dry weight water extract and 20.0±2.4 g GAE/100g 80% (v/v)
323 ethanol extract, respectively. The phenolic content of brown algae harvested from cold water areas
324 such as Danish waters, has shown seasonal dependency and is typically higher in late summer after
325 exposure to UV light during the summer period (can vary from 3 to 10 % phlorotannin of the dry
326 weight) (Connan, Gouæard, Stiger, Deslandes, & Gall, 2004; Parys, Kehraus, Glombitza, Koenig,
327 Pete & Kuepper, 2009). Hence, the difference could be explained by difference in UV exposure
328 between the two types of *F. vesiculosus* due to different locations of harvesting. However, the
329 relative difference between the TPC obtained with the two different extraction solvents is
330 comparable with the observations by Hermund et al. (2015) and Honold et al. (2016).

331

332 The antioxidants properties of the extracts were evaluated and the EC50 values were determined.
333 Both extracts exhibited radical scavenging, metal chelating and reducing power activities (Table 1).
334 EE showed higher radical scavenging activity compared with WE ($p < 0.05$), (EC50 of 3.7±0.1 and

335 4.2±0.2 µL/mL, respectively). In a previous study, high TPC have been correlated with high radical
336 scavenging activity (Wang et al., 2009). On the other hand, no similar correlation was found for the
337 metal chelating ability as no significant difference in their ability to chelate metals was found
338 between EE and WE. Moreover, much higher concentration of extract was needed in order to obtain
339 50% metal chelating activity *in vitro* compared to radical scavenging activity.

340 Prior to the storage trial, the extracts were pooled and freeze dried to produce freeze dried extracts.
341 The dried extracts were analysed for pigments and monophenolic compounds to investigate which
342 compounds could also contribute to the antioxidant properties apart from phlorotannins. Moreover,
343 chlorophylls can work as sensitizers in protooxidation. However, these compounds are not
344 considered a problem in the present study as storage conditions were controlled and the cream was
345 not exposed to any light.

346 Both extracts were visibly colored (brownish). However, only the EE contained pigments in
347 detectable amounts (data not shown). The main pigments were chlorophyll C2, 19-but-fucoxanthin
348 and astaxanthin, which were found in concentrations of 6.3±0.7, 20.3±2.4 and 2.1±0.8 mg per 100g
349 freeze dried EE. Honold et al. (2016) also found that 19-but-fucoxanthin was the most dominant
350 xanthophyll in 80% (v/v) ethanol extract from *F. vesiculosus*. Moreover, similar to the findings in
351 the present study Hermund et al. (2015) did not find fucoxanthin or astaxanthin in the water
352 extracts. However, carotene a and b were found in trace amounts (1.7±0.7 µg/mg dry weight).

353 For the monophenolic compounds only two were detected; phloroglucinol (PG) and *p*-coumeric acid
354 (data not shown). Whereas ethanol extracted both of these phenolic compounds (6.0±3.1 mg PG/g
355 freeze dried extract and 5.6±0.0 mg *p*-coumeric acid/g freeze dried extract), water only extracted
356 phloroglucinol (6.6±3.9 mg PG/g freeze dried extract).

357 The results show that water and ethanol are not efficient in extracting pigments and monophenolic
358 compounds from *F. vesiculosus*. Hence, the target extraction of phlorotannins compromises the

359 extraction of other antioxidant substances. TPC results can be influenced by other reducing agents
360 than phenolic compounds, such as sugars, and therefore these results cannot be dedicated to
361 phlorotannins alone. More advanced analytical approaches for determining the antioxidant
362 contribution of individual phlorotannins would be of high relevance to explain the role of these
363 compounds.

364 365 **3.4. Antioxidant performance of seaweed extracts in facial cream**

366 In order to determine the oxidative stability of the facial cream and the antioxidant performance of
367 the seaweed extracts a storage trial was carried out.

368 The peroxide value measures the formation of lipid hydroperoxides formed during the initial stage
369 of lipid oxidation. The secondary oxidation products are formed when lipid hydroperoxides are
370 further broken down and these compounds are often associated with quality deterioration. For
371 example Salcedo & Nazareno (2015) associated rancidity of almonds with pentanal, hexanal,
372 nonanal and 2-heptenal. These volatiles could therefore be relevant to the facial creams in the
373 present study as they contained almond oil.

374 In order for an antioxidant to be efficient it needs to be able to interact with the lipid oxidation
375 processes. Hence, the antioxidant should either work on preventing the formation of lipid
376 hydroperoxides, e.g. by chelating lipid oxidation catalysts such as metal ions, or the breakdown of
377 these lipid hydroperoxides to secondary oxidation products by working as a radical scavenger and
378 inactivating lipid radicals and terminate the lipid oxidation. However, the location and interfacial
379 properties of the antioxidants have been found to play a major role (Frankel, Huang, Kanner &
380 German, 1994; Alemán, Bou, Guardiola, Durand, Villeneuve, Jacobsen & Sørensen, 2015).

381

382 **3.4.1. Fatty acid composition and tocopherol consumption**

383 The cream consisted of approximately 22% almond oil, which was the main contributor to
384 unsaturated fatty acids, mainly oleic (18:1) and linoleic acid (18:2). Other and more saturated fatty
385 acids came from emulsifiers, MF and VE. On average, the fatty acid composition was as follows
386 (only content $\geq 0.5\%$): 16:0, 11.2%; 16:1 (n-7), 0.5%; 18:0, 10.7%; 18:1 (n-9), 53.3%; 18:2 (n-6),
387 18.0%; 10:2 (n-4), 0.7%; and 20:0, 0.6%. No changes in the fatty acid composition were observed
388 during storage, which suggested that lipid oxidation only happened to a low extent in all samples.
389 Tocopherols which are naturally present in almond oil can also act as antioxidants by scavenging
390 radicals inside the oil droplets in the emulsion. Typically consumption of tocopherols is observed in
391 an emulsion when lipid oxidation occurs.

392 In the present study, four tocopherol homologues were detected in the creams, α -, β -, γ -, and δ -
393 tocopherol. The most abundant was γ -tocopherol (3.9 ± 0.2 mg/g oil), followed by δ - (1.2 ± 0.1 mg/g
394 oil), α - (0.7 ± 0.0 mg/g oil), and β -tocopherol (0.1 ± 0.0 mg/g oil), respectively. The high level of
395 tocopherols in the cream mainly originated from the added vitamin E and almond oil. The total
396 tocopherol content (TTC) was calculated and the consumption rate was determined by the decrease
397 in TTC from day 0 to 42. The consumption rate results show (Table 2) a decrease ($p < 0.05$) in TTC
398 during storage in the control (CON) and in cream with WE added in the high concentration. On the
399 other hand, tocopherol consumption was not observed in cream added low concentration of WE and
400 EE as no significant decrease ($p > 0.05$) was found in TTC from day 0 to 42.

401 In previous studies (Hermund et al, 2015; Honold et al, 2016; Karadag et al., 2017; Poyato et al.,
402 2017) consumption of tocopherols in different food and cosmetic systems have been observed also
403 when extracts from *F. vesiculosus* were added. It was suggested that the tocopherols are the main
404 antioxidants in these systems and responsible for inhibiting lipid oxidation resulting in a decrease in
405 tocopherols during storage. However, when the tocopherols are used up lipid oxidation accelerates.

406 Therefore, regeneration of tocopherols is necessary to maintain antioxidant activity of the
407 tocopherols. Regeneration of tocopherols by polyphenolic compounds have previously been
408 observed, however this regeneration can only occur if the polyphenolic compounds are located at
409 the oil/water interphase. Honold et al. (2016) found that phenolic compounds from *F. vesiculosus*
410 extracted with acetone or ethanol had a higher interfacial affinity compared with phenolic
411 compounds extracted with water. Previous studies have found that ethanol and acetone, or aqueous
412 solutions of these are effective extraction solvents and therefore recommended for extraction of
413 phlorotannins from *F. vesiculosus* (Wang et al., 2009; Farvin & Jacobsen, 2013; Koivikko,
414 Loponen, Honkanen & Jormalainen, 2005). Moreover, other studies have discussed how the
415 polarity of the extraction media extracted different phlorotannins from *F. vesiculosus* and how
416 structure and physical properties affect the behaviour of the phlorotannins in o/w emulsions and
417 efficacy as antioxidants (Karadag et al., 2017; Hermund et al., 2018). Hence, in the present study
418 degradation of tocopherols in samples without seaweed extract could indicate synergistic effect
419 between interfacial phlorotannins and tocopherols, as previous studies observed. However, it cannot
420 be ruled out that the phlorotannins work as antioxidants before tocopherols are used in samples
421 added EE, and that beyond the storage time of the present study, tocopherol consumption would
422 occur. Synergistic effect between ascorbic acid and tocopherol has been found (Niki, 1991;
423 Mäkinen, Kähkönen & Hopia, 2001). Similar synergistic studies between phlorotannins and
424 tocopherols are of interest to study the role of these interfacial phlorotannins in tocopherol-
425 containing emulsion systems such as skin care and food emulsions.

426

427 **3.4.2. Primary oxidation products**

428 In Table 3, the oxidation rate of peroxide value during the first 28 days of storage is shown for the
429 different sample codes.

430 The peroxide value increased significantly in all creams until day 28 ($p < 0.05$). Thereafter some
431 decreased or stayed unchanged until the end of the storage trial. The oxidation rate (%) of the
432 peroxide values for each cream was calculated from day 0 to 28, and the ranking was as follows:
433 WEC1 (87.2%) > Control (84.8%) > WEC2 (59.9%) > EEC2 (53.6%) > EEC1 (41.3%). This
434 indicates that there were antioxidant activity of both WE and EE. However, at low concentrations
435 of WE (WEC1) no activity was found. A decrease in peroxides after day 28 was observed in cream
436 with the highest concentration of seaweed extract (WEC2, EEC2). This decrease could be due to the
437 degradation of primary oxidation products to secondary oxidation products, e.g. by the presence of
438 reductants in the extracts (responsible for the reducing power, Table 1) which can reduce Fe(III) to
439 Fe (II) and promote degradation of hydroperoxides (Jacobsen, Adler-Nissen & Meyer, 1999).

440

441 3.4.3. Secondary oxidation products

442 Ten major volatile compounds associated with oxidation of oleic, linoleic and α -linolenic acid
443 (Guillen & Uriarte, 2012; Poyato, Ansorena, Navarro-Blasco & Astiasarán, 2014), were selected for
444 analysis of the cream: pentanal, hexanal, heptanal, octanal, nonanal, *t*-2-heptenal, *t*-2-octenal, 2-
445 ethyl-1-hexanol, 1-penten,3-one and 2-pentylfuran.

446 Figure 1 shows the concentration of hexanal in the cream during storage. The initial concentration
447 of hexanal was the same in all creams ($p > 0.05$) at day 0. No lag phase was identified. Whereas the
448 control and cream added WE in the low concentration had a significant increase ($p < 0.05$) in
449 hexanal from day 0 to the end of the storage, the hexanal concentration stayed constant in all the
450 other samples. This might imply that the control and cream added WE in the low concentration was
451 less oxidatively stable compared with cream added WE in the high concentration or EE (both
452 concentrations). In a previous study, Hermund et al. (2015) also found no antioxidant effect of WE

453 in low concentration (0.1%, w/w) in mayonnaise. This indicates a concentration dependent efficacy
454 of the extracts.

455 For hexanal and other volatile compounds such as 1-penten-3-one, pentanal, heptanal, and 2-
456 pentylfuran the differences among samples and also more evident variations during storage
457 compared to their initial concentrations was bigger than for octanal, nonanal, *t*-2-heptenal, *t*-2-
458 octenal, 2-ethyl-1-hexanol where no variation could be found between days or storage time (data
459 not shown). Pentanal, hexanal and 2-pentylfuran showed the overall picture of the formation of
460 secondary oxidation products in the cream, and were chosen as representatives of the overall results
461 of the formation of secondary volatile oxidation products during storage. The oxidation rate (%)
462 between day 0 and 42 was calculated for these volatiles in order to have an overview of the
463 antioxidant efficacy of the extracts towards reducing formation of the secondary oxidation products
464 in comparison with the control. The results are shown in Table 3. No significant ($p > 0.05$) increase
465 in formation of hexanal and pentanal was observed in the samples from day 0 to 42 when
466 calculating the consumption rates. However, for 2-pentylfuran the increase was significant in all
467 samples from day 0 to 42 (indicated as *** in Table 3) and samples with seaweed extracts had
468 lower oxidation rates than the control. The lowest oxidation rate for 2-pentyl furan was observed for
469 the sample with the ethanol extract added in the lowest concentration. Poyato et al. (2017) also
470 found no or a negative development in pentanal during storage (20°C, dark) of cream added water
471 or acetone extract. The results show that the development in secondary oxidation is minimal during
472 42d dark storage at room temperature. Acceleration of lipid oxidation using initiators such as
473 synthetic radicals or iron/Fenton oxidants are often used approaches to determine difference in
474 resistance to lipid oxidation of systems containing antioxidants. However, the methods have at this
475 point has not been validated for determination of long-term oxidative stability under normal
476 circumstances. All though, the results in this study indicate that $\text{FeCl}_2/\text{H}_2\text{O}_2$ shows great potential,

477 more studies are needed to confirm this observation. Hence, this leaves one validated option until a
478 full-validation of the initiators ability to reproduce the pattern has conducted, namely that long-term
479 storage stability studies are needed in order to determine the antioxidant efficacy of *F. vesiculosus*
480 extracts towards formation of secondary oxidation products.

481 Beside chemical analysis of volatile compounds, simple sensory observations were performed by
482 DOD (degree of difference) testing on creams day 0, 21 and 42. DOD testing (scale 1 to 5, where 1
483 is no difference and 5 is highly different) was used to compare the control with creams added
484 seaweed extract. No difference (score 1) between creams added WE and the control was observed at
485 day 0. However, the cream containing EE (score 2) was different from these creams. This difference
486 was noted as a slight fishy or seaweed smell, which was also observed at day 21 and 42.

487 Docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) are two omega-3 polyunsaturated
488 fatty acids, which are found in fish, but was also identified in *S. latissima* year round (Marinho,
489 Holdt, Jacobsen & Angelidaki, 2015). A previous study identified volatile compounds from brown
490 seaweed and found among other alcohols and ketones, e.g. 1-penten-3-one (Ferraces-Casais, Lage-
491 Yusty, Rodríguez-Bernaldo de Quirós & López-Hernández, 2013). 1-Penten-3-one has been found
492 as a decomposition product of EPA and DHA giving rise to fishy off-flavors (Venkateshwarlu, Let,
493 Meyer & Jacobsen, 2004). This compound was identified in all creams including the control cream
494 without added seaweed extract. However, a higher concentration of 1-penten-3-one was found in
495 cream containing 0.1% EE compared to the control cream throughout the storage, which could
496 cause the observed difference in the DOD testing. The concentration ranged from 20.0 ± 3.0 to
497 23.6 ± 0.5 ng 1-penten-3-one/g control, and 25.3 ± 0.8 to 35.3 ± 03.9 ng 1-penten-3-one/g EEC2.

498 **3.5. Physical stability of facial cream with added seaweed extracts**

499 When evaluating new ingredients, the physical appearance and quality are important as consumer
500 acceptance is so important. Hence, physical quality parameters such as color changes and emulsion
501 stability were evaluated on all creams at different storage time points.

502 Color was measured at day 0, 21 and 42. The extracts to some extent contained pigments, and
503 especially the EE was visibly brown due to the presence of brown colored xanthophylls, e.g.
504 fucoxanthin, as described previously. Hence, it was expected that the cream would change color
505 when the extracts were added and that the color would be more intense when high concentrations of
506 extracts were used. This was confirmed, as addition of extract generally changed the color of the
507 cream. In Figure 2a the yellowness in the samples at the three time points are shown as an example
508 of these changes. Significantly higher ($p < 0.05$) yellow and red color at day 0 was detected for
509 cream containing WE or EE compared to the control. The yellowness ranged between creams as
510 follows: EEC2 > EEC1 > WEC2 > WEC1 > Control. Moreover, the lightness of the cream
511 decreased when EE was added. These results are in agreement with data reported by Poyato et al.
512 (2017) who also found that the extracts colored the cream more yellow/red, and therefore could not
513 mimic the conventional cream perfectly because of the presence of pigments in the extracts.

514 The size of oil droplets dispersed in the facial cream was determined at day 0 and at the end of
515 storage as shown in Figure 2b. D[3,2] was used as a measure for the droplet size. At day 0 the
516 droplets were significantly bigger ($p < 0.05$) in the control compared to those in cream containing
517 seaweed extracts. This could indicate that the extracts contained surface active compounds, e.g.
518 amphiphilic polyphenolic compounds that were able to reduce the oil droplet size, as suggested by
519 Honold et al. (2016). Whereas, the droplet size in the control and in cream added the low
520 concentration (C1) of WE stayed unchanged during storage, the droplet size increased significantly
521 in creams added EE and WE in high concentration (C2) from approximately 0.8 to 2.5 μm . This

522 high increase in droplet size during storage indicates a degree of destabilization of the emulsion as
523 Poyato et al. (2017) also observed. These changes in droplet size did not affect the lipid oxidation or
524 cause visible instability of the cream. However, the droplet size could perhaps increase more if the
525 storage time was prolonged, and then the destabilization may result in syneresis, which would be an
526 unacceptable quality change to the consumer.

527

528 **4. Conclusions**

529 Different types of extraction solvents produced extracts with different antioxidant properties.

530 Ethanol extract (EE) had a higher TPC, DPPH radical scavenging activity (and reducing power)
531 than the water extract (WE), indicating that TPC is related to radical scavenging activity. No
532 influence of extraction media was found for metal chelating ability.

533 Ethanol also co-extracted more pigments and monophenolic compounds compared to water. The
534 characterization of antioxidants showed that the co-extraction of pigments and monophenolic
535 compounds were limited as only few were identified and quantified. Hence, these compounds most
536 likely only contributed slightly to the overall antioxidant activity.

537 Application of *F. vesiculosus* ethanol and water extracts in facial cream improved the oxidative
538 stability of the facial cream over the storage period of up to 42 days. EE in a concentration of 0.1%
539 (w/w) was the most efficient antioxidant in skin care emulsions. The higher efficacy of EE over WE
540 was related to high phenolic content, high radical scavenging activity, high reducing power, and
541 moderate metal chelating ability.

542 Danish *F. vesiculosus* is a potential source of natural antioxidants, which may be used to reduce
543 lipid oxidation in facial cream and to protect degradation of unsaturated functional lipids in these
544 types of products. Further investigations are needed to confirm long term antioxidant effects of the
545 EE extract in skin care products. In order to address further functional properties of the seaweed

546 extracts, e.g. anti-ageing activity, further characterization of similar extracts should be made and
547 their effect on the skin should be investigated.

548

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553

554 **6. References**

- 555 Alemán, M., Bou, R., Guardiola, F., Durand, E., Villeneuve, P., Jacobsen, C., & Sørensen, A.-D.M.
556 (2015). Antioxidative effect of lipophilized caffeic acid in fish oil enriched mayonnaise and milk.
557 *Food Chemistry*, *167*, 236–244
- 558 AOCS (1998). AOCS Official Method Ce 1b-89 Fatty acid composition by GC. Marine oils. IL,
559 USA: Champaign
- 560 AOCS (2009). Official method Ce 8-89, determination of tocopherols and tocotrienols in vegetable
561 oils and fats by HPLC. IL, USA: Champaign
- 562 Aust, L.B., Garcula Jr., M.C., Beard, S.A., & Washam II, R.W. (1985). Degree of difference test
563 method in sensory evaluation of heterogeneous product types. *Journal of food science*, *50*(2), 511-
564 513
- 565 Bligh, E. G., & Dyer, W. J., (1959). A rapid method for total lipid extraction and purification.
566 *Canadian Journal of Biochemistry and Phycology*, *37*, 911–917
- 567 Chkhikvishvili, I. D., & Ramazanov, Z. M. (2000). Phenolic substances of brown algae and their
568 antioxidant activity. *Applied Biochemistry and Microbiology*, *36*, 289–291

569 Connan, S., Goulard, F., Stiger, V., Deslandes, E., & Gall, E.A. (2004). Interspecific and temporal
570 variation in phlorotannin levels in an assemblage of brown algae. *Botanica Marina*, *47*, 410–416

571 Farvin, K.H.S., Baron, C.P., Nielsen, N., & Jacobsen, C. (2010). Antioxidant activity of yoghurt
572 peptides: Part 1 – in vitro assays and evaluation in n-3 enriched milk. *Food Chemistry*, *123*, 1081–
573 1089

574 Farvin, K.H.S., & Jacobsen, C. (2013). Phenolic compounds and in vitro antioxidant activities of
575 selected species of seaweed from Danish coast. *Food Chemistry*, *138*, 1670–1681

576 Ferraces-Casais, P., Lage-Yusty, M.A., Rodríguez-Bernaldo de Quirós, A., & López-
577 Hernández, J. (2013). Rapid identification of volatile compounds in fresh seaweed. *Talanta*, *115*,
578 798–800

579 Frankel, E.N., Huang, S.W., Kanner, J., & German, J.B. (1994). Interfacial phenomena in the
580 evaluation of antioxidants: mBulk oil vs emulsions. *Journal of Agricultural and Food Chemistry*,
581 *42*, 1054-1059

582 Guillen, M., & Uriarte, P. (2012). Aldehydes contained in edible oils of a very different nature after
583 prolonged heating at frying temperature: Presence of toxic oxygenated alpha, beta unsaturated
584 aldehydes. *Food Chemistry*, *131*, 915–926

585 Hartvigsen, K., Lund, P., Hansen, L.F., & Holmer, G. (2000) Dynamic headspace gas
586 chromatography/mass spectrometry characterization of volatiles produced in fish oil enriched
587 mayonnaise during storage. *Journal of Agricultural and Food Chemistry*, *48*, 4858–4867

588 Hermund, D.B., Yesiltas, B., Honold, P., Jónsdóttir, R., Kristinsson, H.G., & Jacobsen, C. (2015).
589 Characterisation and antioxidant evaluation of Icelandic *F. vesiculosus* extracts in vitro and in fish-
590 oil-enriched milk and mayonnaise. *Journal of Functional Foods*, *19*, 828–841

591 Hermund, D.B., Plaza, M., Turner, C., Jónsdóttir, R., Kristinsson, H.G., Jacobsen, C., & Nielsen,
592 K.F. (2018). Structure dependent antioxidant capacity of phlorotannins from Icelandic *Fucus*
593 *vesiculosus* by UHPLC-DAD-ECD-QTOFMS. *Food Chemistry*, 240, 904–909

594 Holdt, S.L., & Kraan, S., (2011). Bioactive compounds in seaweed: Functional food applications
595 and legislation. *Journal of Applied Phycology*, 23, 543–597

596 Honold, P.J., Jacobsen, C., Jónsdóttir, R., Kristinsson, H.G., & Hermund, D.B. (2016). Potential
597 seaweed-based food ingredients to inhibit lipid oxidation in fish-oil-enriched mayonnaise.
598 *European Food Research and Technology*, 242, 571–584

599 Iverson, S.J., Lang, S.L.C., & Cooper, M.H.(2001). Comparison of the Bligh and Dyer and Folch
600 methods for total lipid determination in a broad range of marine tissue. *Lipids*, 36, 1283–1287

601 Jacobsen, C., Adler-Nissen, J., & Meyer, A.S. (1999). Effect of ascorbic acid on iron release from
602 emulsifier interface and on the oxidative flavor deterioration in fish oil enriched mayonnaise.
603 *Journal of Agricultural and Food Chemistry*, 47, 4917-4926

604 Karadag, A., Hermund, D.B., Jensen, L.H.S., Andersen, U., Jónsdóttir, R., Kristinsson, H.G., &
605 Jacobsen, C. (2017). Oxidative stability and microstructure of 5% fish-oil-enriched granola bars
606 added natural antioxidants derived from brown alga *Fucus vesiculosus*. *European Journal of Lipid*
607 *Science and Technology*, 119, 1500578

608 Koivikko, R., Loponen, J., Honkanen, T., & Jormalainen, V. (2005). Contents of soluble, cell-wall-
609 bound and exuded phlorotannins in the brown alga *Fucus vesiculosus* with implications on their
610 ecological functions. *Journal of Chemical Ecology*, 31, 195–212

611 Kozina, L.S., Borzova, I.V., Arutiunov, V.A., & Ryzhak, G.A. (2013). Role of oxidative stress in
612 skin aging. *Advances in Gerontology*, 3(1), 217-222

613 Lee, D.S., Kang, M.S., Hwang, H.J., Eom, S.H., Yang, J.Y., Lee, M.S., Lee, W.J., Jeon, Y.J., Choi,
614 J.S., Kim, Y.M. (2008). Synergistic effect between dieckol from *Ecklonia stolonifera* and b-lactams

615 against methicillin-resistant *Staphylococcus aureus*. *Biotechnology and Bioprocess Engineering*, 13,
616 758–764

617 Marinho, G.S., Holdt, S.L., Jacobsen, C. & Angelidaki, I. (2015). Lipids and Composition of Fatty
618 Acids of *Saccharina latissima* Cultivated Year-Round in Integrated Multi-Trophic Aquaculture.
619 *Marine Drugs*, 13, 4357-4374

620 Mäkinen, M., Kähkönen, M., & Hopia, A. (2001). Ascorbic acid and ascorbyl palmitate have only
621 minor effects on the formation and decomposition of methyl linolate hydroperoxides. *European*
622 *Journal of Lipid Science and Technology*, 103, 683-687

623 Niki, E. (1991). Action of ascorbic acid as a scavenger of active and stable oxygen radicals.
624 *American Journal of Clinical Nutrition*, 54, 1119-1124

625 Parys, S., Kehraus, S., Glombitza, K.-W., Koenig, G.M., Pete, R., & Kuepper, F.C. (2009).
626 Seasonal variation of polyphenolics in *Ascophyllum nodosum* (Phaeophyceae). *European Journal of*
627 *Phycology*, 44, 331-338

628 Poyato, C., Ansorena, D., Navarro-Blasco, I., & Astiasarán, I., (2014). A novel approach to monitor
629 the oxidation process of different types of heated oils by using chemometric tools. *Food Research*
630 *International*, 57, 152–161

631 Poyato, C., Thomsen, B.R., Hermund, D.B., Ansorena, D., Astiasarán, I., Jónsdóttir, R.,
632 Kristinsson, H.G., & Jacobsen, C. (2017). Antioxidant effect of water and acetone extracts of *Fucus*
633 *vesiculosus* on oxidative stability of skin care emulsions. *European Journal of Lipid Science and*
634 *Technology*, 119, 1600072

635 Rawle, A. (1996). *Basic principles of particle size analysis*. Worcestershire: Malvern Ins. Ltd.

636 Salcedo, C.L. & Nazareno, M.A. (2015). Effect of phenolic compounds on the oxidative stability of
637 ground walnuts and almonds. *RSC Advances*, 5, 45878-45887

638 Shantha N.C., & Decker E.A. (1994). Rapid, sensitive, iron-based spectrophotometric methods for
639 determination of peroxide values of food lipids. *The Journal of AOAC International*, 77, 421–424

640 Sugiura, Y., Matsuda, K., Yamada, Y., Imai, K., Kakinuma, M., & Amano, H. (2008). Radical
641 Scavenging and Hyaluronidase Inhibitory Activities of Phlorotannins from the Edible Brown Alga
642 *Eisenia arborea*. *Food Science and Technology Research*, 14 (6), 595 – 598

643 Venkateshwarlu, G., Let, M.B., Meyer, A.S., & Jacobsen, C. (2004). Chemical and olfactometric
644 characterization of volatile flavor compounds in a fish oil enriched milk emulsion. *Journal of*
645 *Agricultural and Food Chemistry*, 52, 311–317

646 Van Heukelem, L., & Thomas, C.S. (2001). Computer-assisted high-performance liquid
647 chromatography method development with applications to the isolation and analysis of
648 phytoplankton pigments. *Journal of Chromatography. A*, 910, 31–49

649 Wang, T., Jónsdóttir, R., & Ólafsdóttir, G. (2009). Total phenolic compounds, radical scavenging
650 and metal chelating of extracts from Icelandic seaweed. *Food Chemistry*, 116, 240-248

651 Yang, J., Guo, J., & Yuan, J. (2008). *In vitro* antioxidant properties of rutin. *Food Science and*
652 *Technology*, 41, 1060–1066

653