"Antioxidant effect of water and acetone extracts of *Fucus vesiculosus* on oxidative stability of skin care emulsions"

Candelaria Poyato[1], Birgitte Raagaard Thomsen[2], Ditte B. Hermund[2], Diana Ansorena[1], Iciar Astiasarán[1], Rosa Jónsdóttir[3], Hordur G. Kristinsson[3], Charlotte Jacobsen[2]

[1]Department of Nutrition, Food Science and Physiology, Faculty of Pharmacy, University of Navarra, Irunlarrea s/n, 31008-Pamplona, Spain.

[1]IDISNA, Instituto Biosanitario de Navarra

[2]Division of Food Technology, National Food Institute, Technical University of Denmark, DK-2800 Kgs. Lynby, Denmark.

[3]Matís ohf., Vínlandsleid 12, 113 Reykjavík, Iceland

***Corresponding author:** Charlotte Jacobsen

E-mail address: chja@food.dtu.dk

Key words: cosmetic emulsion, brown algae, lipid oxidation, skin care emulsion

Abbreviations: RF, reference; WE, water extract; AE, acetone extract; TTC, total tocopherol content; PV, peroxide value; VC, volatile compounds;

European Journal of Lipid Science and Techonology, 119 (3), 1600072 (2017).

ABSTRACT

A water and an acetone extract of *Fucus vesiculosus* were evaluated as potential natural sources of antioxidant compounds in skin care emulsions. To assess their efficacy in inhibiting lipid oxidation caused by photo- or thermoxidation, they were stored in darkness and room temperature as control conditions, and compared to samples stored under accelerated conditions (light and room temperature, or darkness and 40 °C). The presence of extracts in the skin care emulsions induced remarkable colour changes when the emulsions were exposed to light, and more extensively under high temperature. High temperature also caused greater increments in the droplet size of the emulsions. The analysis of the tocopherol content, peroxide value and volatile compounds during the storage revealed that, whereas both water and acetone extracts showed (at 2 mg/g of emulsion) protective effect against thermooxidation, only the water extract showed antioxidant activity against photooxidation.

1. INTRODUCTION

Natural derived ingredients combined with carrier agents, preservatives, surfactants, humectants and emulsifiers are commonly used in skin care products. A natural ingredient is based on botanically sourced ingredients currently existing in nature (such as herbs, roots, essential oils and flowers), in order to reduce synthetic compounds in the final product. Nowadays, there is an increasing interest in natural ingredients (1) because of the negative perception of the synthetic ones. Thus, the evolution of the cosmetic industry to adapt products to the trends of the XXI century consumer has given rise to new challenges.

Emulsions are the most common type of delivery system used in cosmetics, with creams and lotions being the best-known. Skin care emulsions enable a wide variety of active ingredients to be quickly delivered to skin. In this sense, there are many factors that can potentially influence the physical and oxidative stability of these emulsions, such as fatty acids and ionic composition, type and concentration of antioxidants and prooxidants, emulsion droplet size and interfacial properties (2, 3, 4, 5).

Lipid oxidation can occur in skin care emulsions (6, 7) and can be triggered or enhanced by light and/or high temperatures. Moreover, the high content of vegetable oils in skin care emulsions´ formulations might contribute to induction of lipid oxidation, causing unpleasant odours, colour changes and in consequence, low quality products (6, 7).

Therefore, it is important to limit lipid oxidation and to extend the shelf life of skin care products using natural antioxidants. In addition, some natural antioxidants can give the skin product added functional value. It has recently been suggested that the use of natural antioxidants, such as vitamins A and E, in skin care formulations could provide a preventive therapy for skin photoaging. (8, 9). Moreover, beauty-improving formulations of skin care emulsion with seaweed extracts or micro algae added have also been reported (10).

Vitamin E is one of the most used natural antioxidants in skin care products, usually added due to its radical scavenging activity (9). However, in highly complex matrices containing trace metals, such as cosmetics products (11), other antioxidant properties such as metal chelating ability might be of relevance. Therefore, to stabilise lipid rich skin care products, extra addition of antioxidants might be necessary.

Natural derived antioxidants from various plants and marine algae have shown great potential in improving oxidative stability in these kinds of products. A high variety of bioactive compounds, such as pigments, sulphated polysaccharides, proteins and polyphenols, have been described for different types of brown and red algae by Farvin and Jacobsen (12). Especially, the high content of phlorotannins, the major polyphenolic compounds in brown algae, has been related to high antioxidant activity, as these compounds can work both as radical scavengers and metal chelators (13, 14). Furthermore, phlorotannins have been shown to possess biological activity of potential medicinal value making them valuable in development of nutraceutical, pharmaceutical and cosmetic products (15, 16). Balboa et al. (1) successfully used a *Sargassum muticum* extract to improve the oxidative stability of oil-in-water model emulsions with cosmetic purposes. Farvin and Jacobsen (12) found that, compared to other brown algae, *Fucus vesiculosus* had higher phenolic content and exhibited the highest antioxidant activity *in vitro*. Wang et al. (14, 17) found that the high *in vitro* antioxidant activity of *F. vesiculosus* extracts were related to a high phenolic content and identified the phlorotannin tetramer, fucodiphloroethol E, to be the main contributor to this activity. Moreover, Hermund et al. (18) evaluated *F. vesiculosus* extract as potential antioxidant against lipid oxidation in fish-oil-enriched food emulsions, obtaining promising results.

65 Whereas the *in vitro* antioxidant properties of *F. vesiculosus* have been widely studied
66 (14, 19), applied studies on the antioxidant activity of *F. vesiculosus* extracts to hinder
67 lipid oxidation are sparse (18, 20, 21).
68 The aim of this study was to evaluate the antioxidant properties of two extracts obtained
69 from Icelandic brown algae *F. vesiculosus* (water and acetone extract) in terms of
70 assessing their efficacy to inhibit lipid oxidation during the storage of skin care
71 emulsions, at room temperature in darkness and under two different accelerated
72 conditions (photo- and thermooxidation).
73

2. MATERIAL AND METHODS

2.1. Materials

The ingredients for the formulation of the skin care emulsion were purchased from Urtegaarden (Allingåbro, Denmark). Lanette wax has a composition of C16: 45–55%and C18:45–55% from coconut oil. VE (vegetable emulsifier) is fat from palmeoil and it has a composition of 60–70%monoglycerider, free glycerol 1.5% and free fatty acids 1.5%. All solvents used were of high-performance liquid chromatography (HPLC) grade and purchased from Lab-Scan (Dublin, Ireland). External standards were purchased from Sigma Aldrich (Steinheim, Germany).

2.2. Extraction

The two extracts used in this study were provided by Matís in Iceland and have been used in previous studies (water extract previously used by Hermund (18) and the acetone extract by Honold et al. (21) and both extract by Karadaˬg et al. [19]).

The extractions were carried out according to Wang et al. (14, 17). The seaweed was collected in the Hvassahraun coastal area near Hafnarfjordur, southwestern Iceland, in 2011. At the collecting spot the seaweed was washed with clean seawater to remove salt, epiphytes and sand attached to the surfaces of the samples and transported to the laboratory. The samples were rinsed with tap water and wiped with paper towel. The samples were freeze-dried, pulverised into powder and stored at -80 °C prior to extraction.

The extracts were produced as follows: Five grams of the algal powder was mixed with 100 mL of distilled water or 70 % aqueous acetone (v/v). Hereafter these were incubated on a platform shaker (InnovaTM 2300, New Brunswick Scientific, Edison, NJ) for 24 h at 200 rpm and at room temperature. The mixture was centrifuged at 2168

g for 10 min at 4 °C and filtered with Whatman no. 4 filter paper. Each extraction was conducted in duplicate. The extracts were stored at -80 °C until use.

The water extract (WE) composition was as follows: phenolic content, 14.73 g gallic acid equivalent/100 g extract; chlorophylls, 0.46 µg/mg extract; xanthophylls, 2.17 µg/mg extract; carotenes, 1.72 µg/mg extract; iron, 4.39 µg/mg extract and copper, 0.91 µg/mg extract. The acetone extract (AE) composition was as follows: phenolic content, 18.55 g gallic acid equivalent/100 g extract; chlorophylls, 0.85 µg/mg extract; xanthophylls, 0.75 µg/mg extract; iron, 9.53 µg/mg extract and copper, 1.21 µg/mg extract. The composition of the seaweed extracts was determined and reported in Hermund et al. [18] and Honold et al. [22]. The composition of potential antioxidant or prooxidant compounds was as follows: total phenolic content (g GAE/100 g dry extract): WE, 18.4 ± 0.1, AE, 23.2 ± 1.1, protein (w/w% dry extract): WE, not detected, AE, 2.3 ± 0.0, tocopherol (mg/g dry extract): WE, a: 19.0 ± 1.9, b: 2.9 ± 0.0, g: 6.2 ± 0.2, d: 24.5 ± 1.2, AE, a: 4.0 ± 0.3, b: 1.9 ± 0.7, g: 2.5 ± 0.8, d: 12.9 ± 0.6, iron (mg/g dry extract): WE, 4.4 ± 1.0, AE, 9.5 ± 1.1. Pigments (mg/mg dry extract): chlorophylls: WE, 0.5 ± 0.0, AE: 0.8 ± 0.1, carotenoids: WE, 3.9 ± 0.9, AE: 0.8 ± 0.1. Antioxidant properties (at a concentration of 1.5mg dry extract/mL water) of the two seaweed extracts were as follows: DPPH radical scavenging activity (%): WE, 93.6 ± 0.5, AE, 101.5 ± 0.9, metal chelating ability (%): WE, 75.6 ± 10.8, AE, 28.9 ± 6.7, reducing power (OD700): WE, 0.8 ± 1.1, AE, 1.6 ± 0.1.

2.3. Skin care emulsion production and storage conditions

The two *F. vesiculosus* extracts, water (WE) and acetone extract (AE) were applied to the skin care emulsion in two concentrations (1 and 2 mg/g of skin care emulsion). These amounts were successfully used in a previous experiment in which the stability of a fish-oil-enriched milk and mayonnaise were tested under different storage conditions

(18). Thus, five different types of skin care emulsions were finally obtained: RF (reference, without extract), WE1, WE2, AE1, AE2. Table 1 shows all the ingredients for the fat phase and the aqueous phase (including the extract, when used). These ingredients were weighted in individual pots and heated to 70-75 °C. The oily phase was slowly poured into the water phase under powerful steering (9.500 rpm, Ultra-Turrax® T25basic). After the homogenization process, the emulsions were cooled to room temperature. The skin care emulsions were packed in transparent 50 ml containers. Then the samples were stored under three different conditions: room temperature (21.2±0.7 °C) and darkness (A0), room temperature (24.4±0.3 °C) and light (A+) and high temperature (42.3±1.5 °C) and darkness (H0). The samples were analysed at different storage times (0, 7, 21, 35 and 56 days).

2.4. Lipid extraction

Lipids were extracted from the skin care emulsions according to the method described by Iverson, Lang, and Cooper (22) based on the method of Bligh and Dyer (23). For each sample, two oil extractions were performed and analyzed independently. Resulting lipid extracts were used as starting material for the analysis of peroxides, fatty acid composition and tocopherol content.

2.5. Fatty acid composition (fatty acid methyl esters, FAME)

The fatty acid composition of the oil phases was determined after fatty acid methylation and analysis by GC-FID. The Bligh and Dyer (23) lipid extract from skin care emulsion, corresponding to 30-60 mg lipid, were weighted in vials. 100 µL toluene, 200 µL heptane with 0.01 % (v/v) BHT and 100 µL internal standard (C23:0) (2 % w/v) were added. One mL of BF_3 in methanol was added to the lipid extract mixture and the lipids were methylated in a one-step procedure using a microwave oven (Multiwave3000

SOLV, Anton Paar, Graz, Austria) with a 64MG5 rotor. The settings for the microwave were 5 min at 500 Watt followed by 10 min cooling. The fatty acid methyl esters (FAMEs) were washed with 1 mL saturated NaCl and 0.7 mL heptane with 0.01 % (v/v) BHT. The heptane phase was transferred to a GC vial and FAMEs were analysed by GC (HP 5890A, Agilent Technologies, Palo Alto, CA, USA) according to AOCS (24). For separation DB127-7012 column (10 m x ID 0.1 mm x 0.1 µm film thickness, Agilent Technologies, Palo Alto, CA, USA) was used. Injection volume was 0.2 µL in split mode (1:50). The initial temperature of the GC-oven was 160°C. The temperature was set to increase gradually being as follows: 160 -200 °C (10.6 °C/ min), 200 °C 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 kept at 240 °C for 3.8 min. The measurements were performed at storage day 0 and 56, in duplicates, and the results were given in % of total area.

2.6. Tocopherol content

The lipid extracts from the skin care emulsions were evaporated under nitrogen and dissolved in heptane. The samples were analysed by HPLC (Agilent 1100 Series, Agilent Technology) according to AOCS (24) to quantify the contents of α-, β-, γ- and δ-tocopherols. These tocopherol homologues were separated using a silica column (Waters, Dublin, Ireland, 150 mm, 4.6 mm, 3 µm silica film). A stock solution added 10 mg tocopherols (mixture of α-, β-, γ- and δ-tocopherols) per litre was prepared to determine the retention time of the tocopherols and the peak areas of the given standards. The peak areas of the standard solution were used to calculate the tocopherol content of the samples. The analyses were done in duplicates and results were reported as µg tocopherol/g skin care emulsion.

2.7. Peroxide value (PV)

177 PVs of the lipid extract of the skin care emulsions were determined at all sampling
178 points. This was done according to the method by Shantha and Decker (25), based on
179 the formation of an iron-thiocyanate complex. The coloured complex was measured
180 spectrophotometrically at 500 nm (Shimadzu UV1800, Shimadzu Scientific
181 Instruments, Columbia, MD, USA). The analyses were done in duplicate and the results
182 were expressed in milliequivalents peroxides per kg oil (meq O_2/kg oil). In addition, for
183 every sample, oxidation rates were calculated as follows:

184 $$Oxidation\ rate\ (\%) = \frac{\left(PV_{day35or56} - PV_{day1}\right)}{PV_{day1}} \times 100$$

185

186 **2.8. Volatile compounds (VC)**

187 Tenax GR™ packed tubes were used to collect volatile compounds by dynamic
188 headspace. The collection of the volatile compounds was carried out using 4 g of
189 emulsion (including 30 mg internal stdandard (30 µg/g of 4-methyl-1-pentanol in
190 ethanol)) and 20 mL of distilled water. The volatile secondary oxidation products were
191 collected at 45 °C under purging with nitrogen (flow of 150 mL/min) for 30 min,
192 followed by flushing the Tenax GR™ packed tube with nitrogen (flow of 50 mL/min
193 for 5 min) to remove water. The trapped volatiles were desorbed using an automatic
194 thermal desorber (ATD-400, Perkin- Elmer, Norwalk, CT) connected to an Agilent
195 5890 IIA model gas chromatograph equipped with a HP 5972 mass selective detector.
196 The settings for the MS were: electron ionization mode, 70 eV, mass to charge ratio
197 (*m/z*) scan between 30 and 250 mau. Chromatographic separation of volatile compounds
198 was performed on a DB1701 column (30m× ID 0.25mm× 0.5 µm film thickness, J&W
199 Scientific, Folsom, CA, USA) using helium gas flow (1.3 mL/min).
200 The temperature programme was as follows: 3 min at 35 °C, 3 °C/min from 35 to 120
201 °C, 7 °C/min to 120-160 °C, 15 °C/min 160-200 °C and hold for 4 min at 200 °C.

202 The auto sampler collector setting details were: 9.2 psi, outlet split: 5.0 mL/min,
203 desorption flow: 60 mL/min. The analysis was performed in triplicate in all sampling
204 points and the results were given in ng/g of emulsion.
205 The quantification of the different volatiles was done by the use of a calibration curve
206 prepared from the following external standards dissolved in ethanol: pentanal
207 (calibration range, c.r: 0.007-3.77 mg/g), hexanal (c.r: 0.005-2.69 mg/g), heptanal (c.r:
208 0.008-4.15 mg/g), *trans*-2-heptenal (c.r: 0.005-2.95 mg/g), octanal (c.r.0.006-3.11
209 mg/g), *trans*-2-octenal (c.r: 0.005-2.91), 1-octen-3-ol (c.r: 0.006-3.01 mg/g) and 2-
210 ethyl-1-hexanol (c.r: 0.006-3.19 mg/g). 1 µL of every solution prepared at different
211 concentrations, was added to a Tenax GR™ tube and flushed with nitrogen (flow of 50
212 mL/min for 5 min) to remove the solvent. Then, the volatiles were analyzed in the same
213 way as for the samples. Results for each compound were expressed as ng/g of extract,
214 and oxidation rates were calculated as follows:

215 $$Oxidation\ rate\ (\%) = \frac{\left(VC_{day35or56} - VC_{day1}\right)}{VC_{day1}} \times 100$$

216

217 **2.9. Droplet size distribution**

218 The size of fat globules in the o/w emulsion systems was determined by laser diffraction
219 using a Mastersizer 2000 (Malvern Ins.,Worcestershire, UK).
220 The skin care emulsion was diluted 1:9 in SDS buffer (10 mM NaH_2PO_4, 5 mM SDS,
221 pH 7) prior to analysis. Droplets of the diluted skin care emulsion was added to
222 recirculation water (3000 rpm) reaching an obscuration of 12–14%. The set-up used was
223 the Fraunhofer method, which assumed that all sizes of particles scatter light with the
224 same efficiency and that the particles are opaque and transmits no light. The refractive
225 index (RI) of sunflower oil at 1.469 and water at 1.330 were used as particle and
226 dispersant, respectively. Measurements were performed on day 0 and 56, in triplicates.

227 Results were given as surface area mean diameter D(0.9), which indicates that 90% of
228 the volume of the oil droplets is smaller than this value.
229
230 **2.10. Colour determination**
231 Colour of skin care emulsions was measured using a digital colorimeter (Chromameter-
232 2 CR-200, Minolta, Osaka, Japan) to obtain the colour coordinates L*, a* and b*. These
233 values were used to calculate the euclidean distance value
234 ($\Delta E = \sqrt{\left(L_2^* - L_1^*\right)^2 + \left(a_2^* - a_1^*\right)^2 + \left(b_2^* - b_1^*\right)^2}$) that allowed two types of comparisons:
235 comparison of samples with and without extracts, and also comparison of the initial
236 colour of every sample to that detected along the storage. The measurements were
237 performed in triplicates.
238
239 **2.11. Statistical processing**
240 Mean and standard deviation of results obtained were calculated. For each parameter,
241 one way ANOVA with Tukey-b post hoc multiple comparisons was used in order to
242 evaluate the significant differences among samples and treatments. Within each type of
243 sample, the differences between 0 and 56 days were evaluated by Student t-test.
244 The statistical analysis of data was done using the SPSS 15.0 program (SPSS, INC.,
245 Chicago, IL, USA). Significance level of $p \leq 0.05$ was used for all evaluations.
246

3. RESULTS AND DISCUSSION

3.1. Physical changes

3.1.1. Droplet size determination

The distribution of oil droplets in the skin care emulsions was determined at the beginning and the end of every storage condition (Fig. 1). D(0.9) value was selected to highlight the differences among samples and treatments. This value indicates that 90% of the volume of droplets is smaller than this value. When an increment in this parameter is observed, a destabilization of the emulsion system is occurring. Regarding the accelerated storage conditions, D(0.9) tended to increase in the presence of light (A+), but a significant increase in D(0.9) was only observed at high temperature (H0). Hence, in all emulsions stored at high temperature, these changes caused a large destabilization of the emulsions, leading to an evident syneresis at the end of the storage (visual evaluation). Due to this observed syneresis effect in H0 stored samples at day 56, only samples stored up to 35 days were considered for further analysis in this case. Moreover, WE2 and AE2 showed the highest D(0.9) increments, highlighting that the presence of high doses of these extracts in the skin care emulsions under high temperature could influence the physical stability of the system. In the case of A+ stored samples, only the addition of 2 mg/g of AE extract seemed to decrease the stability of the skin care emulsions, although no syneresis was found.

3.1.2. Colour

In order to evaluate the influence of the presence of some pigments (carotenoids, xantophylls and chlorophylls) on the colour of the emulsions, euclidean distance value (ΔE) was calculated before the storage (day 0). Thus, when comparing colour between the extracts containing samples and the RF at day 0, the calculated ΔE were 3.90, 6.80, 4.93 and 7.59 for WE1, WE2, AE1, and AE2, respectively. All these values were higher

than 2, leading to conclude that clear colour differences were noticed between the extract containing samples and the RF (26), with a strong influence of the concentration and extract type of *F. vesiculosus* used. The instrumental colour data confirmed that, whereas no differences in lightness (L*) and redness (a*) were found, yellowness (b*) was significantly higher ($p<0.05$) in the emulsion containing extract compared to the RF. These colour differences were dose dependent and higher in the AE containing emulsions as compared to WE ones. These colour differences pointed out that the use of seaweed extracts did not perfectly mimic the characteristic of conventional skin care emulsions, due to the presence of several types of pigments.

Additionally, the evolution of the colour was also measured during the storage conditions, and ΔE were also determined (Fig. 2), comparing, in this case, each sample with their own colour at day 0. Results pointed out that the five emulsions did not maintain the colour during storage, as observed by the ΔE increments in all cases. The increment was higher in the samples with higher amount of extract (WE2 and AE2). Particularly light, and mainly temperature (40 °C), induced remarkable colour changes in the samples containing extracts, whereas the lowest colour changes in the RF sample at high temperature was observed. These changes were a consequence of an increment in a* value and a reduction in the L* value (data not shown), as the samples became more brown over time. The storage conditions could induce oxidative reactions that might affect pigments such as fucuxanthin and chlorophylls and produce colour changes in the samples. However, this deserves more research.

3.2. Oxidative changes

Almond oil (*Prunus amygdalus*) is one of the most valuable skin care oils due to its penetrating, moisturising and restructuring properties, and high content of unsaturated fatty acids. It can be used for numerous skin problems because of their anti-

299 inflammatory, emollient, sclerosant and cicatrizing effects (27). Therefore, in the
300 present study, almond oil was used in the formulation of the oil-in-water skin care
301 emulsions. However, the susceptibility of the unsaturated lipids present in almond oil to
302 oxidation might be a major cause of quality deterioration and rancidity in the skin care
303 emulsion. The lipid profile of the samples was determined at the beginning and at the
304 end of the storage in every sample, and it was observed that it remained unchanged
305 during the storage period (data not shown), with oleic acid as the major fatty acid,
306 followed by linoleic, and the saturated ones, palmitic and stearic acid. On average, the
307 fatty acid composition was as follows: 14:0, 0.27%; 16:0, 12.0%; 16:1 (ω-7), 0.11%;
308 18:0, 12.1%; 18:1 (ω-9), 53.8%; 18:1 (ω-7), 0.66%; 18:2 (ω-6), 18.4%; 18:3 (ω-3),
309 0.08%; 20:0, 0.28%; 20:1 (ω-11), 0.47%; 20:4 (ω-6), 0.31%; 22:1 (ω-9), 0.17%.
310
311 *3.2.1. Tocopherol content during storage*
312 Four tocopherol homologues were detected in the skin care emulsions, α-, β-, γ-, δ-
313 tocopherol. The most abundant one at the beginning of the storage was gamma-
314 tocopherol (4895±151 μg tocopherol/g skin care emulsion) followed by delta- (1657±94
315 μg tocopherol/g skin care emulsion), alpha- (1329±72 μg tocopherol/g skin care
316 emulsion) and beta-tocopherol (116±10 μg tocopherol/g skin care emulsion),
317 respectively. The addition of seaweed extract did not affect the content of tocopherols.
318 Similar changes were observed in the four homologues, so the sum of all of them was
319 calculated, and represented as the total tocopherol content (TTC) along the storage (Fig
320 3). The TTC decreased in all samples during storage, and the highest rate of decrease
321 was observed at the high temperature conditions (H0).
322 It is worthy to highlight, that the AE showed the highest tocopherol protective effect at
323 all storage conditions, with AE2 being the best concentration. However, WE showed

protective effects only when exposed to light (up to 50 days for WE1) and high temperature storage conditions (up to 20 and 35 days for WE1 and WE2, respectively). This protective effect of WE and AE on tocopherols could be due to a synergistic effect between tocopherol and phenolic compounds or pigments, contributing to the regeneration of tocopherol in skin care emulsions containing extracts.

3.2.2. Peroxide value (PV)

The autoxidation of unsaturated fatty acids is a chain process occurring autocatalytically through free radical intermediates, and it can be accelerated during storage by exposure to light, temperature and in presence of redox metals. On that basis, the primary oxidation compounds, expressed as the peroxide content of the skin care emulsions stored in the different conditions, were determined (Table 2).

At the beginning of the storage, WE2 and AE2 samples showed slightly higher PV values than RF samples ($p<0.05$). This could be a consequence of the presence of trace metals in the algae extracts (iron and copper) which promoted, together with the temperature of processing (70-75 °C), oxidative reactions at an initial stage. During storage, significant increments in PV were found in all samples ($p<0.05$).

At A0 storage conditions an increase in the oxidation rate, between day 1 and 56, was found in WE2 (151%) and AE2 (154%) compared to RF (108%). It is well known that interactions between lipid hydroperoxides and transition metals acts as precursors of lipid oxidation compounds. Consequently, metal chelating capacity is claimed as one of the important mechanisms of antioxidant activity. (2, 28). Regarding this, several studies showed that *F. vesiculosus* extracts, containing phlorotannins, had good ferrous ion-chelating capacity (12, 17). In this sense, the presence of phlorotannins may form complexes with metals and inactivate their catalytic effects in promoting peroxide decomposition. Due to this antioxidant effect, an accumulation of peroxide compounds

350 in the extract containing samples might take place and consequently lead to a lower
351 formation of volatile compounds, as will be discussed below.
352 Regarding A+, after 56 days of storage, while samples containing AE showed the
353 highest (AE2) or not significant differences (AE1) on PV, both WE samples had lower
354 PV than RF one ($p<0.05$). This could be due to the higher content of carotenoids in WE
355 , as carotenoids are well known inhibitors of free radical chain reactions caused by
356 photooxidation process (29).
357 Moreover, in the case of high temperature conditions it should be pointed out that a
358 higher oxidation rate, between day 1 and 35, was found in RF (185%), compared to
359 WE2 (102%) and AE2 (110%). The high content of polyphenols in the extracts, with
360 radical scavenger activity could interfere in the lipid oxidation process and thereby slow
361 down fatty acid degradation.
362 *3.2.3. Volatile compounds*
363 Odour deterioration of lipid containing products is caused mainly by the presence of
364 volatile lipid oxidation products, which have an impact on odour at extremely low
365 concentrations. Compounds formed from decomposition of peroxides during storage
366 can either react with unsaturated lipids to form stable and innocuous alcohols, or
367 undergo fragmentations into aldehydes and ketones causing rancidity in unsaturated
368 matrices (30). Major volatile compounds identified from the headspace of the fifteen
369 samples throughout the storage were: four alkanals (pentanal, hexanal, heptanal and
370 octanal), two alkenals (*trans*-2-heptenal and 2-octenal) and two alcohols (1-octen-3-ol
371 and 2-ethyl-1-hexanol). These compounds represent groups of secondary oxidation
372 products resulting mainly from the autooxidation of oleic, linoleic and α-linolenic acid
373 (31, 32, 33). Hexanal and 2-octenal showed the highest initial concentrations (248±99
374 and 222±52 ng/g emulsion, respectively). However, others such as pentanal and
375 heptanal showed greater differences among samples and also more evident variations

during storage compared to their initial concentrations. This was the reason why they were selected to follow their evolution during the whole storage (Fig. 4). During accelerated storage conditions (A+ and H0), the peroxides decomposition generated higher volatile amounts than in the A0 stored samples, so there was a higher transformation rate from hydroperoxides to secondary oxidation products due to thermo- and photooxidation processes. Furthermore, results showed that temperature had significantly higher effect than light on the formation of volatile compounds, with higher absolute amounts of both aldehydes at the end of the storage.

Regarding the presence of extracts, the concentration of pentanal and heptanal varied between skin care emulsions at day 0 and during the storage. On one hand the highest amounts of extracts contributed to increase the pentanal and heptanal concentration at the beginning of all storages conditions. This could be because of the presence of these compounds in the extract itself. Hermund et al., (18) observed higher amounts of some volatile compounds (1-penten-3-ol and 1-penten-3-one) in milk emulsions containing *Fucus vesiculosus* extracts.

On the other hand, in the samples with the highest extract content (WE2 and AE2), pentanal showed significantly lower concentrations in all samples compared to RF at the end of the storage (reduction up to 72% in AE2 samples at A0 storage conditions), whereas heptanal amount was lower than RF only at the end of storage at high temperature (19% reduction). On the other hand, the presence of antioxidant modified the timing of volatile formation. Thus, even though the presence of extract at the beginning of the storage resulted in higher amounts of pentanal and heptanal in all samples, lower oxidation rates were observed during storage in these samples.

In particular, in the light stored samples, lower oxidation rates for pentanal and heptanal were found in WE2 (6.7%, 69%, respectively) and AE2 (-35.2%, 40%, respectively) compared to RF (144%, 211%, respectively). Moreover, at high temperature, AE2

showed the best results against the formation of pentanal and heptanal, with oxidation rates of 261% and 281%, respectively, compared to the rates calculated for RF samples (1251% and 1419%). Finally, it is worth noticing that at A0 stored conditions, while RF samples showed an increment of pentanal (81%) and heptanal (71%) between day 1 and 56, the highest extract concentration samples lead to a reduction compared to their initial amounts. These results were in agreement with the accumulative effect observed in PV in these samples. The presence of the extract decreased the hydroperoxide decomposition rate to volatile compounds at all storage conditions, with AE2 being the most efficient extract. This information helps to elucidate the antioxidant mechanism of those extracts, which may influence the protection of the peroxides decomposition to secondary oxidation products. However, more studies are needed to confirm these findings.

4. CONCLUSIONS

The type of antioxidant extract was a key factor in controlling oxidation processes of skin care products influenced by light or temperature. Whereas both water and acetone extracts of *Fucus vesiculosus* showed (at 2 mg/g of emulsion) protective effect against thermooxidation, only the water extract showed antioxidant activity against photooxidation. Therefore, both the polyphenols content (radical scavenging activity) and, in particular, the presence of phlorotannins (iron-chelating capacity), contributed to decreasing the lipid oxidation. Moreover, the higher carotenoids content in the water extract could inhibit free radical chain reactions caused by the photooxidation process. *Fucus vesiculosus* extracts, containing polyphenols, were effective in protecting highly-unsaturated skin care emulsions but gave rise to colour changes particularly when stored in light or at high temperature.

5. ACKNOWLEDGEMENTS

We thank PIUNA (Plan de Investigación de la Universidad de Navarra) and Ministerio de Economía y Competitividad (AGL2014-52636-P and Red de Excelencia Consolider-PROCARSE AGL2014-51742-REDC) for their contribution to the financial support of this work. C. Poyato is grateful to ‘‘Asociación de Amigos de la Universidad de Navarra’’ for the grants received.

6. REFERENCES

(1) Balboa, E. M., Soto, M. L., Nogueira, D. R., González-López, N., Conde, E., Moure, A., Vinardell, M. P., Mitjans, M., Domínguez, H., Potential of antioxidant extracts produced by aqueous processing of renewable resources for the formulation of cosmetics. Ind. Crop. Prod. 2014, 58, 104-110.

(2) McClements,D., Decker, E., Lipid oxidation in oil-in-water emulsions: Impact of molecular environment on chemical reactions in heterogeneous food systems. J Food Sci. 2000, 65, 1270-1282.

(3) Raikos, V., Effect of heat treatment on milk protein functionality at emulsion interfaces. A review. Food Hydrocoll. 2010, 24, 259-265.

(4) Naji, S., Karazhiyan, H., Effect of thermal treatments on functional properties of cress seed (*Lepidium sativum*) and xanthan gums: A comparative study. Food Hydrocoll. 2012, 28, 75-81.

(5) Xu, D., Wang, X., Jiang, J., Yuan, F., Gao, Y., Impact of whey protein - Beet pectin conjugation on the physicochemical stability of beta-carotene emulsions. Food Hydrocoll. 2012, 28, 258-266.

(6) Thanonkaew, A., Wongyai, S., Decker, E. A., McClements, D. J., Formation, antioxidant property and oxidative stability of cold pressed rice bran oil emulsion. J. Food Sci. Tech. 2015, 52, 6520-6528.

(7) Thomsen, B.R., Horn, A.F., Hyldig, G., Taylor, R., Blenkiron, P. & Jacobsen, C., Investigation of lipid oxidation in high and low fat skin care products: case-study on lip and cleanser formulations. Manuscript in preparation, 2015.

(8) Briganti, S., Picardo, M., Antioxidant activity, lipid peroxidation and skin diseases. What's new. J. Eur. Acad. Dermatol. Venereol. 2003, 17, 663-669.

(9) Mukherjee, P. K., Maity, N., Nema, N. K., Sarkar, B. K., Bioactive compounds from natural resources against skin aging. Phytomedicine. 2011, 19, 64-73.

(10) Gudmundsdottir, A. B., Omarsdottir, S., Brynjolfsdottir, A., Paulsen, B. S., Olafsdottir, E. S., Freysdottir, J., Exopolysaccharides from *Cyanobacterium aponinum* from the Blue Lagoon in Iceland increase IL-10 secretion by human dendritic cells and their ability to reduce the IL-17^{+}RORγt^{+}/IL-10^{+}FoxP3^{+} ratio in $CD4^{+}$ T cells. Immunol. Lett. 2015, 163, 157–162.

(11) Borowska, S., Brzóska, M. M., Metal in cosmetics: implications for human health. J. Appl. Toxicol. 2015, 35, 551-572.

(12) Farvin, K. H. S., Jacobsen, C., Phenolic compounds and antioxidant activities of selected species of seaweeds from Danish coast. Food Chem. 2013, 138, 1670-1681.

(13) Chkhikvishvili, I. D., Ramazanov, Z. M., Phenolic substances of brown algae and their antioxidant activity. Appl. Biochem. Microbiol. 2000, 36, 336-338.

(14) Wang, T., Jonsdottir, R., Kristinsson, H. G., Thorkelsson, G., Jacobsen, C., Hamaguchi, P. Y., Olafsdottir, G., Inhibition of haemoglobin-mediated lipid oxidation in washed cod muscle and cod protein isolates by *Fucus vesiculosus* extract and fractions. Food Chem. 2010, 123, 321-330.

(15) Mancini, J., Novoa, A. V., Gonzalez, A. E. B., de Andrade-Wartha, E. R. S., Silva, M. D. E., Pinto, J. R., Mancini, D. A. P., Free Phenolic Acids from the Seaweed *Halimeda monile* with Antioxidant Effect Protecting against Liver Injury. J. Biosciences. 2009, 64(9-10), 657-663.

(16) Thomas, N. V.; Kim, S. K., Beneficial Effects of Marine Algal Compounds in Cosmeceuticals. Marine Drugs. 2013, 11, 146-164.

(17) Wang, T., Jónsdóttir, R., Ólafsdóttir, G., Total phenolic compounds, radical scavenging and metal chelation of extracts from Icelandic seaweeds. Food Chem. 2009, 116, 240-248.

(18) Hermund, D. B., Yeşiltaş, B., Honold, P., Jónsdóttir, R., Kristinsson, H. G., Jacobsen, C., Characterisation and antioxidant evaluation of Icelandic *F. vesiculosus* extracts in vitro and in fish-oil-enriched milk and mayonnaise. J. Funct. Food. 2015, 19, Part B, 828-841.

(19) Farvin, K. H. S., Jacobsen, C., Antioxidant Activity of Seaweed Extracts: In Vitro Assays, Evaluation in 5 % Fish Oil-in-Water Emulsions and Characterization. J. Am. Oil Chem. Soc. 2015, 92, 571-587.

(20) Habeebullah, S. F. K., Nielsen, N. S., Jacobsen, C., Antioxidant Activity of Potato Peel Extracts in a Fish-Rapeseed Oil Mixture and in Oil-in-Water Emulsions. J. Am. Oil Chem. Soc. 2010, 87, 1319-1332.

(21) Honold, J. P.; Jacobsen, C.; Jónsdóttir, R.; Kristinsson, H. G.; Hermund, D. B., Potential seaweed-based food ingredients to inhibit lipid oxidation in fish-oil-enriched mayonnaise. Eur. Food Res. Technol. 2015, 1-14.

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

(23) Bligh, E. G.; Dyer, W. J., A Rapid method of total lipid extraction and purification. Canadian Journal of Biochemistry and Physiology 1959, 37, 911-917.

(24) AOCS. AOCS official method Ce 1b-89 fatty acid composition by GC. Marine oils. AOCS Press, Champaign, IL, USA (1998)

(25) Shantha, N. C.; Decker, E. A., Rapid, sensitive, iron-based spectrophotometric methods for determination of peroxide values of food lipids. Journal of AOAC International. 1994, 77, 421-424.

(26) Francis, F.J., & Clydesdale, F.M., Food colorimetry: theory and application. Chapman & Hall, New York (USA) 1975.

(27) Ahmad, Z., The uses and properties of almond oil. Complement. Ther. Clin. Pract. 2010, 16, 10-12.

(28) Let,M. B., Jacobsen, C., Meyer, A. S., Ascorbyl palmitate, gamma-tocopherol, and EDTA affect lipid oxidation in fish oil enriched salad dressing differently. J Agric Food Chem. 2007, 55, 2369-2375.

(29) Stahl, W., Sies, H., Antioxidant activity of carotenoids. Mol. Aspects. Med. 2003, 24, 345-351.

(30) Frankel, E., Lipid oxidation. The oily press LTD, Dundee (Scotland) 1998.

(31) Belitz, Grosch, Schieberle, Lipids. Food Chemistry, Springer-Verlag, Berlin Heidelberg. 2009, pp. 158-247.

(32) Guillen, M., Uriarte, P., Aldehydes contained in edible oils of a very different nature after prolonged heating at frying temperature: Presence of toxic oxygenated alpha, beta unsaturated aldehydes. Food Chem. 2012, 131, 915-926.

(33) Poyato, C., Ansorena, D., Navarro-Blasco, I., Astiasarán, I., A novel approach to monitor the oxidation process of different types of heated oils by using chemometric tools. Food Res Int. 2014, 57, 152-161.

526 Table 1. Formulation of the different samples.

527

Water phase	
Demineralized water	52.5 g/ 100 g
Aloe vera water	10.0 g/ 100 g
Glycerin	6.3 g/ 100 g
MF fat	3.6 g/ 100 g
Natriumbenzoat	0.6 g/ 100 g
F. vesiculosus extract (water or acetonic)	1 or 2 mg/g
Oily phase	
Almond oil	21.8 g/ 100 g
Lanette wax	2.0 g/ 100 g
VE fat	1.8 g/ 100 g
Vitamin E	0.9 g/ 100 g

528

529

Table 2. Effect of adding water or acetonic *F. vesiculosus* extract on PV (meq O_2/kg oil) of cosmetic emulsions under accelerated stored conditions or room temperature and dark. (RF, reference; WE1, water extract (1 mg/g); WE2, water extract (2 mg/g); AE1, acetone extract (1 mg/g); AE2, acetone extract (2 mg/g); S.C, storage condition; A0, darkness and room temperature; A+, light and room temperature; H0, darkness and 40 °C.)

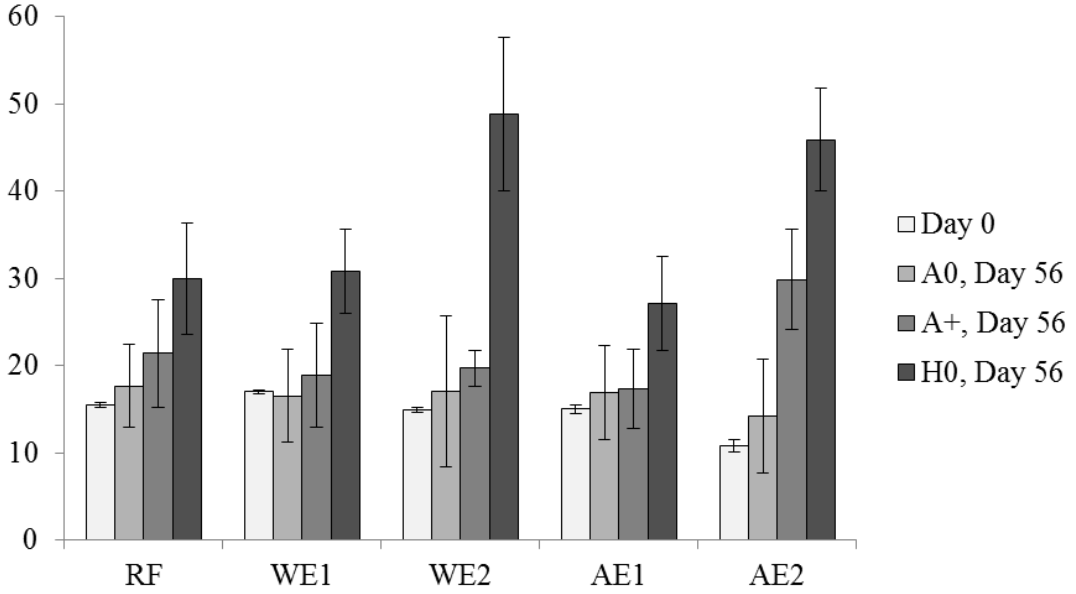
			Peroxide value (meq O_2/kg oil)			
		S.C				
	Day 0		Day 7	Day 21	Day 35	Day 56
RF	5.52 ± 0.51^{A}	A0	6.85 ± 0.29^{Ba}	7.31 ± 1.00^{Aa}	9.48 ± 0.45^{Aa}	11.62 ± 0.31^{A}
		A+	8.22 ± 0.52^{Ab}	11.71 ± 1.07^{Bb}	13.11 ± 1.19^{ABb}	18.93 ± 0.63^{B}***
		H0	11.49 ± 0.84^{ABc}	11.65 ± 0.29^{Ab}	15.01 ± 0.78^{ABb}	
WE1	5.19 ± 0.29^{A}	A0	6.09 ± 0.04^{ABa}	6.97 ± 0.45^{Aa}	8.53 ± 0.36^{Aa}	10.77 ± 1.25^{A}
		A+	8.11 ± 0.05^{Ab}	10.58 ± 0.59^{ABb}	12.37 ± 0.70^{ABb}	15.67 ± 1.29^{A}**
		H0	10.11 ± 1.11^{Ac}	13.08 ± 1.33^{Ac}	14.31 ± 0.07^{Ac}	
WE2	6.58 ± 0.69^{B}	A0	12.89 ± 0.67^{Da}	11.95 ± 0.22^{Ba}	13.96 ± 0.32^{Ca}	17.67 ± 1.38^{C}
		A+	14.08 ± 0.21^{Da}	12.40 ± 0.69^{Ba}	14.38 ± 1.28^{Ba}	$16.42 \pm 1.42^{A\ ns}$
		H0	13.05 ± 1.60^{BCa}	11.38 ± 0.19^{Aa}	13.97 ± 0.19^{Aa}	
AE1	6.28 ± 0.65^{B}	A0	14.26 ± 0.29^{Ea}	13.36 ± 1.03^{Bb}	15.34 ± 0.35^{Db}	18.78 ± 0.71^{C}
		A+	13.16 ± 0.32^{Ca}	10.90 ± 1.15^{ABa}	10.34 ± 0.52^{Aa}	23.97 ± 0.49^{C}***
		H0	13.73 ± 1.71^{Ca}	16.58 ± 1.61^{Bc}	16.47 ± 1.73^{Bb}	
AE2	6.63 ± 0.75^{B}	A0	11.31 ± 0.28^{Cb}	17.03 ± 0.47^{Cb}	12.36 ± 0.87^{Ba}	15.14 ± 0.95^{B}
		A+	9.58 ± 0.23^{Ba}	9.43 ± 0.35^{Aa}	17.36 ± 1.99^{Cb}	18.89 ± 0.55^{B}**
		H0	10.94 ± 0.21^{ABb}	10.43 ± 0.78^{Aa}	14.05 ± 1.05^{ABab}	

Different capital letters in the same column denote significant differences between samples for each storage condition ($p<0.05$)

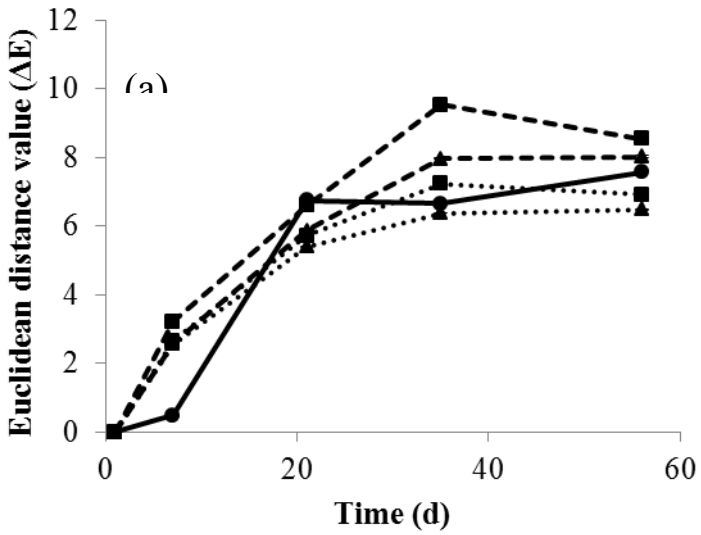
Different small letters in the same column denote significant differences among storage conditions for each sample ($p<0.05$)

*Level of significance for the Student t test comparing storage conditions at day 56: ns = not significant ($p>0.05$); * $p<0.05$; ** $p<0.01$; *** $p<0.001$.*

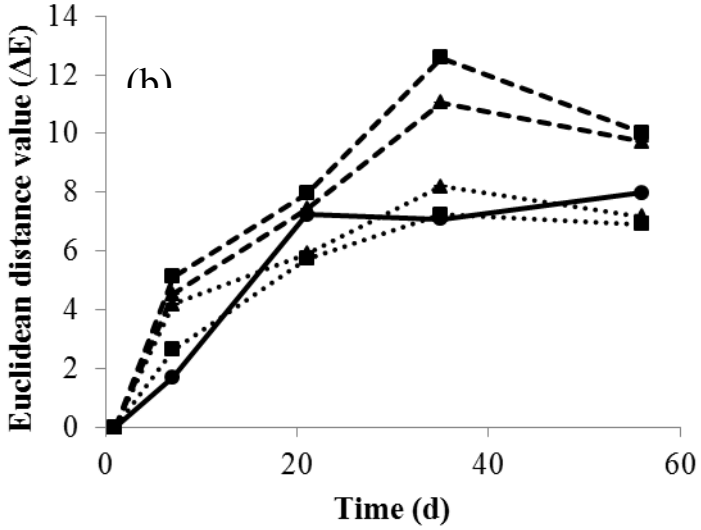
Figure 1. Droplet size distribution on cosmetic emulsions with or without *F. vesiculosus* extract (water or acetonic) after 56 days of storage under accelerated conditions or at room temperature and dark. (RF, reference; WE1, water extract (1 mg/g); WE2, water extract (2 mg/g); AE1, acetone extract (1 mg/g); AE2, acetone extract (2 mg/g); A0, darkness and room temperature; A+, light and room temperature; H0, darkness and high temperature)



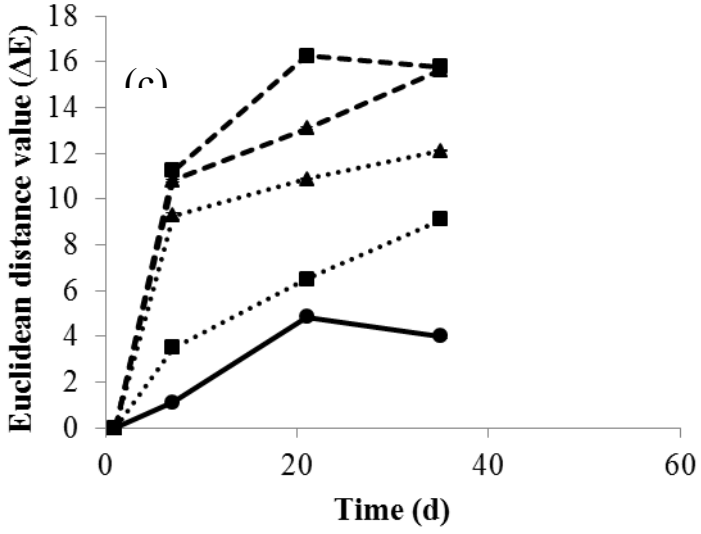
553 Figure 2. Euclidean distance value of the cosmetic emulsions calculated along the
554 storage: it compares color at each time of storage to its color at day 0. (a) A0, room
555 temperature and darkness; (b) A+, room temperature and light; (c) H0, high temperature
556 and darkness. (RF, reference; WE1, water extract (1 mg/g); WE2, water extract (2
557 mg/g); AE1, acetone extract (1 mg/g); AE2, acetone extract (2 mg/g). Error bars
558 indicate SD of the measurements.



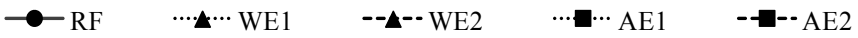
559



560

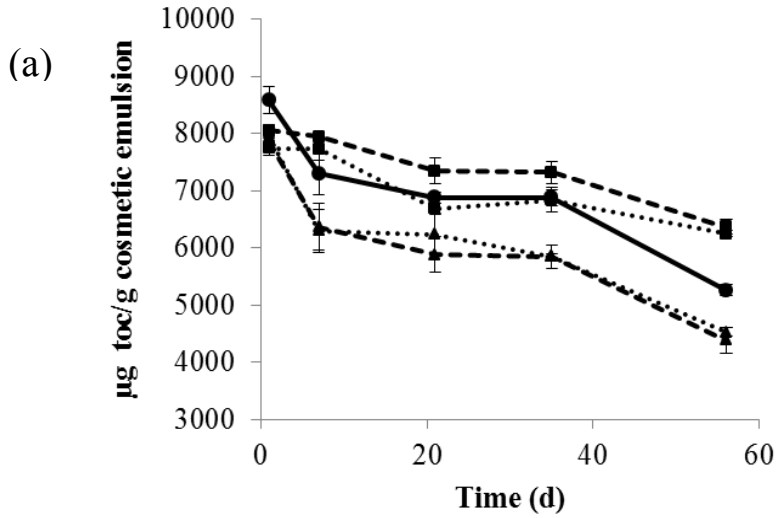


561

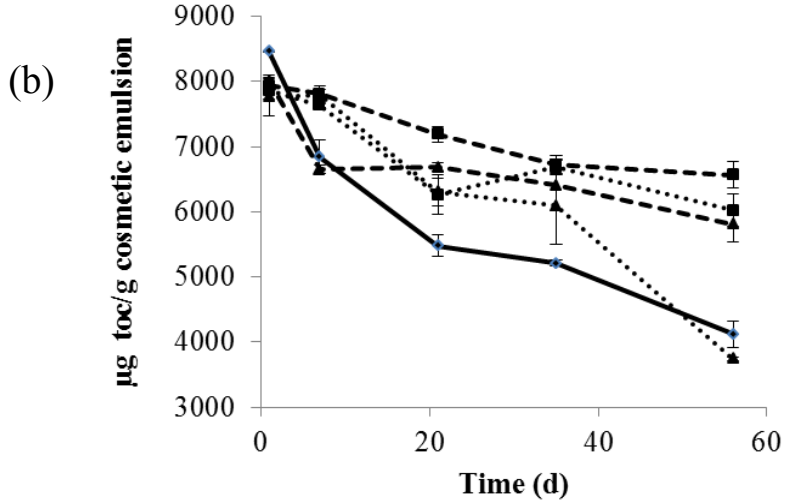


562

563 Figure 3. Total tocopherol content (µg tocopherol/g cosmetic emulsion) in emulsions with WE
564 or AE including a control without any extract during storage. (a) A0, room temperature and
565 darkness; (b) A+, room temperature and light; (c) H0, high temperature and darkness. (RF,
566 reference; WE1, water extract (1 mg/g); WE2, water extract (2 mg/g); AE1, acetone extract (1
567 mg/g); AE2, acetone extract (2 mg/g). Error bars indicate SD of the measurements.

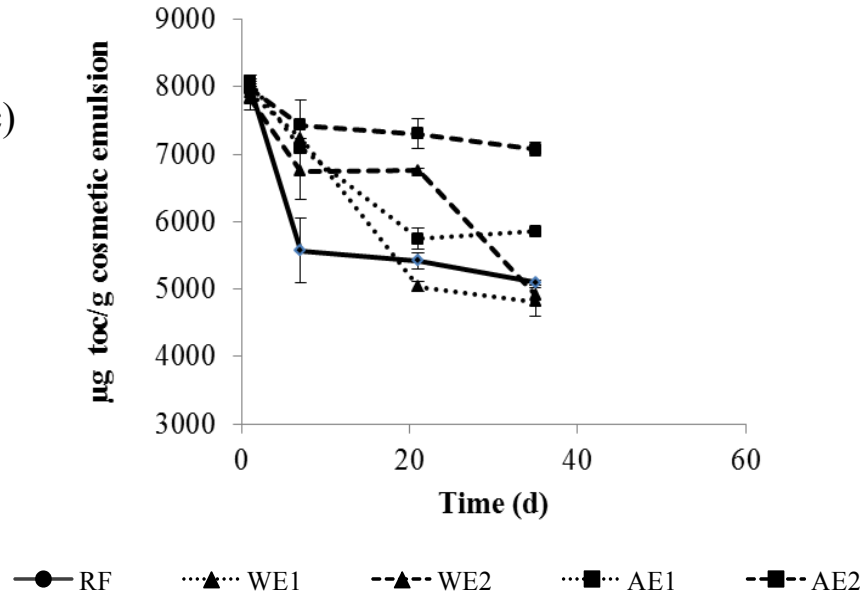


568



569

(c)



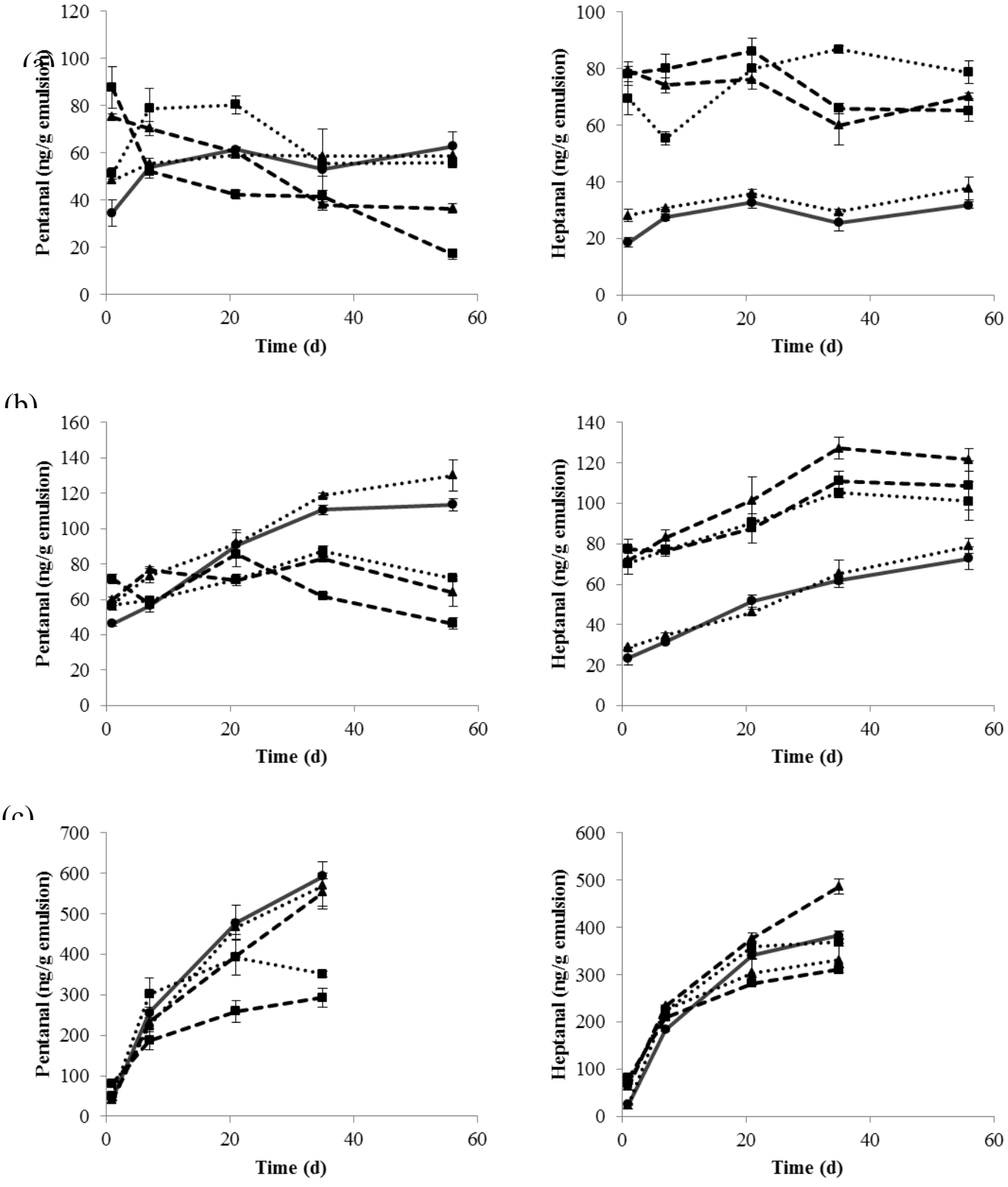
570

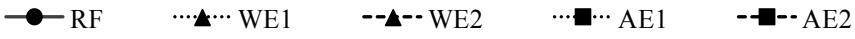
571

572

573

Figure 4. Development of volatile compounds, pentanal and heptanal (ng/g emulsion), during the storage. (a) A0, room temperature and darkness; (b) A+, room temperature and light; (c) H0, high temperature and darkness. (RF, reference; WE1, water extract (1 mg/g); WE2, water extract (2 mg/g); AE1, acetone extract (1 mg/g); AE2, acetone extract (2 mg/g). Error bars indicate SD of the measurements.





579

580