Postprint of Food Chemistry Volume 295, 15 October 2019, Pages 101-109

DOI: https://doi.org/10.1016/j.foodchem.2019.05.092

Cooking effects on bioaccessibility of chlorophyll pigments of the main edible seaweeds

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ABSTRACT

Edible seaweeds are highly consumed food with a rich chlorophyll profile. Although seaweeds are mainly cooked ingested, the influence of cooking on the chlorophyll bioaccessibility remains unknown. In this research, cooked Nori, Sea Lettuce and Kombu were subjected to an *in vitro* digestion and following micellarization investigations. The processing of red seaweed does not affect the chlorophyll recovery, while cooking green and brown seaweeds implies an important

increase in chlorophyll recovery after *in vitro* digestion. In this line, while cooking affects negatively the micellarization rate of chlorophyll derivatives in Nori and Kombu, it does not modify the micellarization in Sea Lettuce. Generally, the chlorophyll bioaccessibility of microwaved seaweeds is always higher than that of boiled ones. However, cooking improves the chlorophyll bioaccessibility in brown seaweeds, while decreases in red seaweeds. In conclusion, the characteristics of food matrix are the determinant factor on the chlorophyll bioaccessibility of cooked seaweeds.

Keywords: chlorophyll bioaccessibility; cooking seaweeds; *in vitro* digestion; Nori; Kombu; Sea Lettuce

1. Introduction

Chlorophyll pigments, probably the most abundant natural pigments in the world (Mysliwa-Kurdziel, & Solymosi, 2016), have showed important bioactive properties, such as antimutagenic and antigenotoxic activities (Negishi, Rai, & Hayatsu, 1997; Simonich et al., 2007). In addition, chlorophyll pigments are strong natural antioxidants acting as free radical scavengers (Lanfer-Marquez, Barros, & Sinnecker, 2005). Specifically, it has been estimated that a common diet implies the ingestion of 26-86 mg of chlorophyll pigments everyday according to a nine-year follow-up epidemical study. The investigation has revealed an elevated risk of colon cancer in men with decreasing intake of chlorophyll pigments (Balder et al., 2006). In spite of the daily consumption of chlorophylls, investigations about the bioaccessibility or bioavailability of these phytochemicals are scarce and limited to higher plants (green

vegetables and fruits), such as spinach leaves and peas (Ferruzzi, Failla, & Schwartz, 2001; Gallardo-Guerrero, Gandul-Rojas, & Mínguez-Mosquera, 2008). More specifically, they are focused on the main chlorophyll pigments in higher plants: magnesium-containing phytylated derivatives which mean chlorophyll *a* and chlorophyll *b*, and tiny amounts of pheophytin and pheophorbide derivatives in plant tissue (Fig. 1).

Actually, marine edible seaweeds, traditionally consumed in Asian countries, have gained popularity in western society due to their reservoir of bioactive compounds such as vitamins, edible fibers, proteins including all the essential amino acids, essential fatty acids with nutritionally ideal n-6/n-3 fatty acid ratios and minerals etc. (Shahidi, 2009; Pangestuti, & Kim, 2011). It was estimated that the annual seaweed production in 2016 was over 30 million tons (FAO, 2016) and this number grows 6% annually, which values $400 a ton per dry weight in the market (Buschmann et al., 2017). The chlorophyll profile of the main edible seaweeds is characterized (Lin et al., 2011; Ferraces-Casais, Lage-Yusty, Rodríguez-Bernaldo de Quirós, & López-Hernández, 2012; Fujii et al., 2012) in relation with the content of *a*, *b* and *c* chlorophyll and pheophytins. But a comprehensive study (Chen, Ríos, Pérez-Gálvez, & Roca, 2017) has showed edible seaweeds contains additional dephytylated (pheophorbide) and oxidative structures (13^2-hydroxy derivatives and 15^1-hydroxy-lactone derivatives). Investigations of chlorophyll bioavailability from edible seaweeds (Nori, Sea Lettuce and Kombu) showed the grade of bioaccessibility or bioavailability is highly related with seaweed species and involves many reactions

including pheophytinization (replacement of magnesium with two hydro atoms), oxidation and newly discovered pheophorbidation reactions which remove the phytol chain from pheophytin to yield pheophorbide under the acid condition of digestion (Chen, & Roca, 2018a; 2018b) (Fig. 1).

Truly, edible seaweeds are always consumed after cooking process to improve the palatability of the product (García-Sartal, Romarís-Hortas, Moreda-Piñeiro, Dominguez-Gonzalez, & Bermejo-Barrera, 2011). During cooking process, chlorophyll pigments in edible seaweeds were modified differently according to different species and cooking conditions (Chen, & Roca, 2018c). Pheophytinization, oxidation and decarboxymethylation reactions are the main reactions influencing the chlorophyll profile of cooked seaweeds, and the final chlorophyll composition in cooked Nori, Sea Lettuce and Kombu seaweeds depends of their own characteristic. Currently, the impact of the cooking procedure has been assessed in other bioavailable fractions from seaweeds (Domínguez-González, Romarís-Hortas, García-Sartal, Moreda-Piñeiro, Barciela-Alonso, & Bermejo-Barrera, 2010; Romarís-Hortas, García-Sartal, Barciela-Alonso, Domínguez-González, Moreda-Piñeiro, & Bermejo-Barrera, 2011) but not in the chlorophyll fraction.

Researches related with the influence of cooking on the bioavailability or bioaccessibility of chlorophyll pigments reveal differences of food matrix. Chlorophyll pigments in heated spinach puree show lower micellarization rate compared with fresh spinach puree, which means less chlorophyll pigments are ready for the intestinal absorption, resulted in a reduced bioaccessibility (Ferruzzi et al.,

2001). While for pea researches, after cooking, more chlorophyll pigments are recovered from the *in vitro* digestion and meanwhile, chlorophyll pigments after cooking process are better micellarizated and cell absorbed (Gallardo-Guerrero et al., 2008), signifying cooking enhances the bioaccessibility and bioavailability of chlorophyll pigments in peas.

In this paper, cooked seaweeds (Nori, Sea Lettuce and Kombu) were subjected to an *in vitro* digestion and following micellarization process. The hypothesis considered in the present study is that cooking of seaweeds increases the bioaccessibility of chlorophyll pigments. To which extend and whether it is similar or not among the three taxa of seaweed is the aim to achieve.

2. Materials and methods

All the following procedures were carried out under green light to avoid the photooxidation of chlorophyll pigments.

2.1. Raw material

Nori (*Porphyra umbilicales*) and Kombu (*Laminaria ochroleuca*) were provided by Algamar (Pontevedra, Spain) while Sea Lettuce (*Ulva* sp.) was provided by Suralgae (Cádiz, Spain). The three macroalgae species were collected on the Atlantic littoral region on the south western part (Cádiz) and the north western part (Pontevedra) of Spain. The dried material (25-45ºC for 30-45 hours) is supplied in vacuum sealed bags.

2.2. Chemicals and reagents

N,*N*-dimethylformamide (DMF) PAR grade and LC/MS grade solvents and water

were supplied by Panreac (Barcelona, Spain), while acetone HPLC grade was supplied by Merck. The deionised water used was obtained from a Milli-Q 50 system (Millipore Corp., Milford, MA, USA). Sodium chloride, α-amylase (porcine pancreas, VI-B), pepsin (porcine), bile extract (porcine), lipase pancreatic (porcine), tetrabutylammonium acetate, ammonium acetate (98%), and butylated hydroxytoluene (BHT) were provided by Sigma-Aldrich Chemical Co. (Madrid, Spain). Pheophorbide *a* and chlorophyll *a* were purchased from Wako Chemicals (Tokyo, Japan). Other reagents (acetone andpotassium chloride analysis grade) were supplied by Teknokroma (Barcelona, Spain).

2.3. Preparation of cooked samples

The procedure is detailed in Chen et al. (2018a) and briefly consists on the combination of 15 g of fresh dried seaweeds (Nori, Sea Lettuce and Kombu) with saline solution in a ratio of 1:20 (w/v). Then, one portion of seaweeds (15g /300 mL saline solution) was maintained raw, and the other two (2×15g /300 mL saline solution) were subjected to heating procedure, boiling (100 ºC for 20 min) and microwave heating (800 W for 15 min), respectively. After that, heated samples were cooled on ice to room temperature and supplemented with de-ionized water to achieve the same water level as before. Finally, all the seaweeds were submitted to homogenizing to obtain the consistent puree and aliquots of 0.1 g of dry seaweeds for Sea Lettuce and 0.2 g of dry seaweeds for Nori and Kombu were stored with nitrogen at -20^{o}C before used. A complete description of the methodology followed is outlined in Fig. S1.

133 *2.4. In vitro digestion*

134 Three edible seaweeds including Nori, Sea Lettuce and Kombu were subjected to
135 the *in vitro* digestion protocol established by Garret, Failla, & Sarama (1999) and
136 modified in detail in Chen et al. (2018a). Each tube of defrosted seaweed material was
137 supplemented with 80 µL of sunflower oil to mimic the daily food matrix in human
138 diet (around 4%). The protocol briefly consists on simulated oral, gastric and
139 intestinal phase of the digestion process. Oral phase consisted in an incubation of
140 α-amylase (2041 U/g) in saline solution (140 mM NaCl, 5 mM KCl, pH 7.0) during
141 10 min at 37^{o}C. Following, the gastric phase was performed at pH 2.0 with a final
142 concentration of 2.4 mg/mL pepsin during 1 h at 37^{o}C. After readjusting the pH to 6.0
143 to initiate the intestinal phase, the incubation required during 2 h a solution with bile
144 salts (2.4 mg/mL), pancreatin (0.4 mg/mL) and lipase (0.2 mg/mL) dissolved in
145 $NaHCO_3$ (0.1 M). Aliquots (2 × 5 mL) of digesta were collected, and placed in -20^{o} C
146 blanketed with nitrogen until analysis.

147 *2.5. Micellarization process*

148 The aqueous micellar fraction (AMF) was obtained by centrifugation of the rest of
149 the digesta at 50000 g at 4^{o} C for 90 min (Beckman model L7-65 Ultracentrifuge) to
150 remove the solid compound and the upper oil droplets. After a careful collection of
151 the AMF, it was filtrated (0.2 µm) to remove interfering aggregates. Aliquots (2 × 5
152 mL) of AMF were used for the chlorophyll analysis after storage of blanketed samples
153 with nitrogen at -20^{o} C.

154 *2.6. Pigment extraction*

All the samples were lyophilized during 12h at -63^{o}C (Virtis, Benchtop K). Next, 200 µL distilled water was added and shaken for 5 min. Then, 200 µL DMF was added and samples were shaken for 5 min. Finally, 1600 µL acetone (0.2% BHT) was added and ultrasonicated (10 min, 720 W). The organic layer was analyzed by HPLC after filtration.

2.7. Pigment identification and quantification by HPLC-UV-Visible

Hewlett-Packard HP 1100 liquid chromatograph system was used to separate and quantify chlorophyll pigments, equipped with a LC HP ChemStation (Rev.A.05.04) and a Mediterranea Sea18 column (reversed-phase, 200×4.6 mm, 3 µm particle size, Teknokroma, Barcelona, Spain) with a guard column (10×4.6 mm). The eluting procedure was described previously by Chen et al. (2017), being the column temperature 25^{o} C, the volume injection 20 µL, and the flow-rate 1.25 mL/min. The on-line UV-Visible spectra were collected from 350 to 800 nm with the photodiode-array detector and sequential detection was recorded at 410, 430, 450 and 666 nm. Identification of chlorophyll derivatives was performed by co-chromatography with chlorophyll standards and from their spectral traits (Chen et al., 2017), except for the chlorin e_6 and oxidized pheophorbide c_1 derivatives whose tentative identification was made by their spectral and polarity characteristics (Mínguez-Mosquera & Gandul-Rojas, 1995; Garrido, Otero, Maestro, & Zapata, 2000). Quantification of chlorophyll derivatives was performed by their respective calibration curves and the calibration equations were obtained by least-squares linear regression analysis over the concentration range of chlorophyll pigments observed in

the analyzed samples. Injections were made twice for five different volumes at each standard solution.

2.8. Statistical analysis

For the investigation of seaweed digestion, at least 4 independent experiments were carried out for each sample, and analysis of each digesta or AMF sample was made in duplicate.

The percentage of recovery rate of chlorophyll derivatives was calculated as nanograms of derivatives in digesta ×100/ that in cooked seaweeds, which provides information of which proportion of chlorophyll pigments present in the cooked seaweeds ready for the following micellarization process. The percentage of micellarization rate was calculated as nanograms of chlorophyll derivatives in AMF ×100/ that in digesta, which gives information of which proportion of chlorophyll pigments in the digesta are ready for the enterocyte uptake. The processing bioaccessibility index was calculated as nanograms of chlorophylls in AMF ×100/ that in the initial fresh dried seaweeds, which gives information of which proportion of chlorophyll pigments in the starting fresh dried seaweed are ready for the enterocyte uptake. Data comparison was performed by One-way analysis of variance (ANOVA, StatSoft, Inc., 2001).

3. Result and discussion

3.1. Stability of chlorophyll pigments after in vitro digestion of cooked seaweeds

Fig. 1 illustrates chlorophyll structures and the main reactions involved during *in vitro* digestion of cooked edible seaweeds, while Table 1 shows the percentage

changes previous and post *in vitro* digestion of cooked Nori, Sea Lettuce and Kombu seaweeds.

The processing of Nori seaweeds does not affect the chlorophyll reactions that proceed during the *in vitro* digestion of this seaweed, independently if they are boiled or microwaved. During the *in vitro* digestion of boiled and microwaved Nori, percentages of total pheophorbides increase significantly ($p<0.01$) with a similar decreasing extent in their respective total pheophytins; the level of 13^2-hydroxy derivatives is not modified while that of 15^1-hydroxy lactone derivatives increases; percentages of pyro derivatives decrease, as well as purpurin-18 *a* derivatives. Interestingly, all these reactions were developed exactly at the same level as during the *in vitro* digestion of fresh dried (not cooked) Nori seaweeds (Chen et al., 2018a). The only difference is the formation of pyropheophytin *a* that reaches higher values after the *in vitro* digestion of processed Nori (6%) than that for fresh dried Nori (3.4%, Chen et al., 2018a), as pyro-derivatives are favored during the cooking of this seaweed (Chen et al., 2018c). The similar stability of chlorophylls during the *in vitro* digestion of fresh and cooked Nori seaweeds ,is mainly due to the resistance of red seaweeds to the cooking process, where the chlorophyll profile is almost unalterable during the boiling and microwaving processing (Chen et al., 2018c). Similar results were also found in the assay of arsenic analysis of Nori seaweeds where arsenic chromatographic profile was the same before and after cooking procedure (García-Sartal, Barciela-Alonso, & Bermejo-Barrera, 2012).

The main characteristic of boiled and microwaved Sea Lettuce, which contains *a*

221 and *b* series of chlorophyll, is the presence of low amounts of chlorophyll *b* (16%)
222 and no chlorophyll *a*, in comparison with fresh dried Sea Lettuce (approx. 75% of
223 chlorophylls, Chen et al., 2018c). In this sense, the main reaction associated with
224 chlorophylls during the *in vitro* digestion is the substitution of the central magnesium
225 by hydrogen (Ferruzzi et al., 2001) but in cooked green seaweeds the substrate of this
226 reaction is limited to the low presence of chlorophyll *b*. Consequently, during the *in*
227 *vitro* digestion of cooked Sea Lettuce, the reactions are strengthened over
228 pheophorbides and pheophytins and not with chlorophylls as that during the i*n vitro*
229 digestion of fresh dried green seaweeds (Chen et al., 2018a). For example, during the
230 *in vitro* digestion of fresh dried Sea Lettuce, the amounts of pheophorbide were not
231 modified, but during the *in vitro* digestion of cooked Sea Lettuce percentages of
232 pheophorbide *a* increased, and more significantly ($p<0.01$) in boiled Sea Lettuce than
233 in microwaved ones. Another important difference is that the oxidative reaction, really
234 important during the *in vitro* digestion of fresh green seaweed (with increases of 9%,
235 Chen et al., 2018a), in cooked Sea Lettuce is less important. 15^1-hydroxy-lactone
236 derivatives remain the same, and percentages of both pyro and purpurin-18 *a*
237 derivatives decline with no phytyl-purpurin-18 *a* detected after *in vitro* digestion, in a
238 similar way to the *in vitro* digestion of fresh green seaweeds (Chen et al., 2018a). In
239 cooked Sea Lettuce, no big differences of pigment stability were found between *a* and
240 *b* series with ratio *a*/*b* similar before and after *in vitro* digestion.

241 The processing of brown seaweeds introduces smooth modifications in the
242 chlorophyll profile (Chen et al., 2018c). Specifically, the cooking of Kombu seaweeds

implies a slight decrease in the percentage of chlorophylls (10%) and the respective increase in the proportion of pheophorbides. This trend strengthens the effect of the *in vitro* digestion on the chlorophyll profile of brown seaweeds. During the *in vitro* digestion of fresh dried Kombu, the chlorophyll profile experiences a decrease on chlorophylls (to 3%) and pheophytins (to 61% of the profile) in favor of pheophorbide proportion (reaching the 36% of the total chlorophylls, Chen et al., 2018a). Consequently, if we combine the reactions over the chlorophylls that proceed during the cooking and *in vitro* digestion of brown seaweeds (Table 1), the chlorophyll profile after the *in vitro* digestion of processed Kombu implies a seaweed even richer in pheophorbide (42% of the total chlorophyll fraction) due to the decrease in chlorophylls (less than 1%) and pheophytins (less than 60%). It is also important to highlight that Kombu as brown seaweed contains chlorophyll derivatives of *a* and *c* series, and the important differences in the structure of both series (Fig. 1) determine the extension of the above-mentioned reactions. Firstly, the lack of phytol in chlorophyll *c* series only permits the transformation into pheophorbide due to the acidic environment during the *in vitro* digestion. And secondly, the higher resistance of *c* series in comparison with *a* series to the *in vitro* digestion (Chen et al., 2018a) establishes the importance of the reactions as with fresh seaweeds: chlorophyll *a* is completely degraded during the *in vitro* digestion while chlorophyll *c* is still present in the digesta of processed Kombu and the proportional increase in pheophorbides is more obvious for *a* series (23%) than for *c* series (13%). 13^2-hydroxy derivatives increase significantly from 9% to 24% ($p<0.01$) for boiled Kombu and from 10% to

25% for microwaved Kombu, respectively. Reverse to Nori and Sea Lettuce, the percentage of 15^1-hydroxy lactone derivatives decreases slightly, mainly due to the degradation of 15^1-hydroxy lactone chlorophyll *a*, while pyro derivatives do not degrade but even increase in boiled Kombu sample. Purpruin-18 *a* derivatives degrade completely after *in vitro* digestion.

In previous studies, it was showed that chlorophyll *b* (Gallardo-Guerrero et al., 2008; Gandul-Rojas et al., 2009; Chen et al., 2018a) and chlorophyll *c* (Chen et al., 2018a) were more resistant than chlorophyll *a* during the pheophytinization reactions. This trend continued under the acid condition of *in vitro* digestion of cooked seaweeds, as chlorophyll *a* was completely degraded or transformed into pheophytin *a* derivatives while low amounts of chlorophyll *b* and chlorophyll *c* remained after *in vitro* digestion.

Consistently, pheophorbidation reactions that remove phytyl chain from pheophytin to yield pheophorbide (Sievers, & Hynninen, 1977; Fig.1) have been also observed during the *in vitro* digestion of cooked samples as that in Nori and Kombu fresh dried ones (Chen et al., 2018a). In cooked Nori and Kombu, obvious proportional increase of pheophorbide *a* series is accounted for the decrease of pheophytin *a* series. The difference resides in Sea Lettuce. While during the *in vitro* digestion of this fresh dried seaweed no pheophorbidation reaction accounts (Chen et al., 2018a), when the digestion uses cooked Sea Lettuce as substrate, the pheophorbidation reaction is active. This may be due to the chlorophyll transformations induced by cooking process, as it has been established (Sievers et al.,

1977; Chen et al., 2018a) that pheophorbidation requires more severe acid condition compared with pheophytinization and the latter is more favored. In fresh dried Sea Lettuce, the main chlorophyll pigments are chlorophyll *a* and chlorophyll *b* series (74% in total, Table 2 in Chen et al., 2018a), while in cooked Sea Lettuce, are pheophytin *a* and pheophytin *b* series (74% in total, Table 1) where the pheophorbidation occurs. However, the occurrence of chlorophyll *b* series (16% and 4%, previous and post digestion) accounts for the less extent of pheophorbidation compared with cooked Nori and Kombu samples. Anyhow, pheophorbidation does not occur for pheophytin *b* series to yield pheophorbide *b* indicating the more stability of pheophytin *b* compared with pheophytin *a*.

Oxidation reactions show different pattern with different cooked seaweeds which are reflected by the changes of oxidative derivatives except for purpurin-18 *a* derivatives that represent consistent decreasing pattern in all the *in vitro* cooked seaweeds, even with a complete degradation in agreement with their chemical properties (Wang, Yin, & Yang, 2013). The formation of 15^1-hydroxy lactone pheophorbide *a* in cooked Nori and Kombu seaweeds after *in vitro* digestion and the increase of 13^2-hydroxy derivatives in Sea Lettuce and Kombu samples, which was also found in their fresh dried counterparts (Chen et al., 2018a), indicates that oxidation reactions are favored in the digestion environment. The degree of oxidation reactions and changes of oxidative derivatives are related with chlorophyll molecules and environmental factors (Ferruzzi, Böhm, Courtney, & Schwartz, 2002; Aparicio-Ruiz, Mínguez-Mosquera, & Gandul-Rojas, 2010) including seaweed

309 endogenous antioxidants that lead to differences among seaweed species in other
310 derivatives including 13^2-hydroxy, 15^1-hydroxy lactone and pyro derivatives. In
311 accordance with that observed in fresh dried Sea Lettuce and Kombu (Chen et al.,
312 2018a), for magnesium-containing chlorophylls (chlorophyll *a*, chlorophyll *b* and
313 chlorophyll *c*), oxidation reactions occur prior to pheophytinization, and then yielded
314 oxidized derivatives are pheophytinizated.

315 In general, the grade of cooking of the seaweed does not introduce serious
316 modifications in the chlorophyll profile during the *in vitro* digestion, similar with
317 previous findings. During the *in vitro* digestion of spinach puree (Ferruzzi et al., 2001)
318 and pea (Gallardo-Guerrero et al., 2008), processing effect on the chlorophyll profile
319 of the digesta is not significant except that additional processing depresses the
320 formation of allomerized chlorophyll derivatives compared with the fresh raw
321 material.

322 *3.2. Recovery rate of chlorophyll pigments after in vitro digestion*

323 Fig. 2 shows the chlorophyll pigments recovered from the digesta that means
324 which proportion of chlorophyll pigments are ready for the following micellarization
325 process. For cooked Nori, the recovery rate is around 80% for the boiled (BN) and 76%
326 for the microwaved samples (MN) and differences between cooked styles are not
327 significant. For Sea Lettuce, cooked samples show significant differences with the
328 recovery rate of total chlorophyll for boiled (BS) and microwaved (MS) samples
329 being around 45% and 74%, respectively. For cooked Kombu samples, a recovery rate
330 of around 50% for both boiled and microwaved samples was achieved, with no

differences, unlike that of Sea Lettuce. Interestingly, the recovery rate of chlorophylls from processed Nori after the *in vitr*o digestion is similar to that from fresh dried Nori, around 75% (Chen et al., 2018a). The red seaweed is, in terms of chlorophyll metabolites, the more resistant to the cooking process (Chen et al., 2018c), nearly without any net degradation of chlorophylls or any modification in the chlorophyll profile during the processing (microwave or boiling). Consequently, as the seaweed is not affected by the cooking process, the recovery rate is the same after the *in vitro* digestion of fresh or processed Nori. On the contrary, the cooking process improves greatly the recovery of chlorophylls during the *in vitro* digestion of the other two seaweeds. Fresh Sea Lettuce presented a recovery rate in the digesta of around 38% of chlorophylls while Kombu as low as 15% (Chen et al., 2018a). The less degradation of chlorophyll compounds during the *in vitro* digestion of processed green and brown seaweeds can be justified partially by the different chlorophyll profile between fresh and cooked seaweeds. Fresh Sea Lettuce and Kombu presents a rich profile of chlorophyll (Chen et al., 2017) while the cooking process introduces modifications in the chlorophyll profile as the transformation from chlorophylls into pheophytins and pheophorbides occurs. It has been shown that chlorophylls are more labile to the *in vitro* digestion than pheophytins (Gandul-Rojas et al., 2009). Consequently, when the initial food matrix is rich in chlorophylls (fresh green and brown seaweeds) the degradation of chlorophyll fraction is higher during the digestion than cooked green and brown seaweeds that are rich in pheophytins and pheophorbides. Previous studies have shown that processing of raw material improves the chlorophylls recovery. In

this sense, the recovery rate of cooked seaweeds in our study is similar as that in cooked pea (82-87%, Gallardo-Guerrero et al., 2008). Several hypotheses have been postulated to explain the protection of chlorophylls during the *in vitro* digestion from processed foods: from the possibility of the inactivation of chlorophyll degradative enzymes during the cooking to the fact that cooking could disrupts cell walls and organelle membranes facilitating greater access of digestive enzymes to substrate (Thakkar, Maziya-Dixon, Dixon, & Failla, 2007). However, the different extracellular material explains the differences between seaweeds. Previous investigations related with the culinary treatment of edible seaweeds revealed as well the promoted recovery of substances including protein and amino acid (Maehre, Edvinsen, Eilertsen, & Elvevoll, 2016), arsenic fractions (García-Sartal et al., 2012) etc.

In any case, the most interesting result is the different influence of the processing technique in the recovery of chlorophylls in green seaweeds (Fig. 2). It was clear that in contrast to the other seaweeds, microwave treatment resulted in a smoother technique than boiling just for green seaweeds. In support of this result, during the cooking process of green seaweeds, it has been shown that microwave degraded only 14% of total chlorophylls while boiling reached almost 30% (Chen et al., 2018c). It is clear that the effect of the different processing techniques in the percentage of recovery is mediated by the cellular structure of the different seaweeds.

3.3. Micellarization rate of chlorophyll pigments from cooked seaweeds

Fig. 3 shows the micellarization rate of cooked Nori, Sea Lettuce and Kombu samples showing the ability of chlorophyll derivatives to be incorporated into mixed

micelles and potentially absorbed by intestinal cells. Boiled and microwaved Nori presents 16% and 18% of total chlorophyll micellarization rate respectively; while different cooked Sea Lettuce shows identical micellarization rate (10%); and boiled and microwaved Kombu, 15% and 22% respectively, with significant differences. Interestingly, the processing of green seaweeds does not introduce any modification in the micellarization rate, being the same as in fresh seaweeds (Chen et al., 2018a) and the lowest. A difference, in red and brown seaweeds boiling processing decreases significantly the percentage of micellarization while microwaved seaweeds exhibit similar rates as in fresh raw material (Chen et al., 2018a). In conclusion, boiling processing seems to be more drastic in the micellarization of chlorophylls while microwave treatment allows higher micellarization rates. In this line, it has been found that heating process decreased the micellarization percentage of a wide range of carotenoids such as β-carotene, lutein and astaxanthin during the *in vitro* digestion of spinach, broccoli, savoy cabbage and salmon etc. (Ferruzzi et al., 2001; O'Sullivan, Karen, Aherne, & O'Brien, 2010; Chitchumroonchokchai, & Failla, 2017), while the micellarization process is also associated with food matrix or digestion environment which makes the influence of thermal process not obvious or even positive (Liu, Bi, Xiao, & Mcclements, 2015; Dhuique-Mayer, Servent, Descalzo, Mouquet-Rivier, Amiot, & Achir, 2016). Red and brown algae hold the largest amount of polysaccharides, which always show ion binding and sorption ability (Davis, Volesky, & Mucci, 2003; Lahaye, & Robic, 2007). Meanwhile, these compounds could be released or easily accessible after thermal process (Melo, Feitosa, Freitas, & Paula,

2002; Rodriguez-Jasso, Mussatto, Pastrana, Aguilar, & Teixeira, 2011). It is possible that the excess polysaccharides (in red and brown seaweeds) in digestive system promoted by boiling interact with bile salts or other compounds thus impair the micellarization of chlorophyll pigments.

The study of the influence of cooking on the micellarization process of chlorophyll derivatives have shown contradictory results. Due to the importance of bile salts in the micellarization process of chlorophyll pigments and carotenoids (Garrett et al., 1999; Ferruzzi et al., 2001), processing techniques can promote or depress the micellar formation of chlorophyll pigments according to different food matrix. In the research of chlorophyll pigments in spinach puree (Ferruzzi et al., 2001) where the chlorophyll profile in micellar fractions was mainly pheophytins, it was found that cooking decreased the micellarization rate of hydrophobic pheophytins by improving the release of food components during thermal processing such as mineral and fiber that could interact with bile salts thus depress the micellarization of pheophytin. At difference, in the research of chlorophyll pigment in peas (Gallardo-Guerrero et al., 2008), it had been found that cooking and bleaching improve the micellarization of chlorophyll pigments with the explanation that the de-esterification of the methyl groups in the pectin caused by heating weakens the interaction of pectin and bile salts. It can be implied from those results that the influence of cooking on the micellarization rate of chlorophyll derivatives varies with specific food matrix. The results obtained in the present investigation also support such statement, for green seaweeds the cooking process has no effect in the

419 chlorophyll micellarization rate, but in brown and red seaweeds boiling decreases the
420 percentage of chlorophyll micellarization.

421 Consistently, independently of the processed state and seaweed species,
422 pheophorbides have higher micellarization rate than pheophytins in each sample (Fig.
423 3), mainly due to the hydrophilic nature of pheophorbides (Gandul-Rojas et al., 2009).
424 The influence of the polarity overpasses the fact that processing affects negatively the
425 micellarization of pheophorbides independently of seaweeds or style of cooking. In
426 addition, in cooked Sea Lettuce, *a* series are more favored than *b* series, as also
427 reflected in other investigations with different food matrix (Ferruzzi et al., 2001;
428 Gandul-Rojas et al., 2009) while in cooked Kombu, differences between *a* series and
429 *c* series are not obvious (to the best of knowledge, no previous data are available).

430 *3.4. Chlorophyll characterization in AMF of cooked seaweeds after in vitro digestion*

431 After *in vitro* digestion and micellarization process, AMF from cooked seaweeds
432 were obtained and analyzed for the chlorophyll profile (Table 2). AMF from fresh
433 dried seaweeds were characterized mainly by an increase in the percentage of
434 pheophorbide and a high percentage of oxidative chlorophyll derivatives in
435 comparison with their respective digesta (Chen et al., 2018b). Both principles also
436 characterized the AMF from cooked seaweeds, but mediatized by the processing of
437 the seaweeds. Effectively, the AMF of the seaweeds are enriched in pheophorbide, in
438 spite of the lower micellarization rates of pheophorbide from cooked seaweeds. Even
439 in green and brown seaweeds, the percentage of pheophorbide from AMF of cooked
440 seaweeds is higher than that from fresh dried seaweeds.

441 The comparison of oxidized chlorophyll percentages (13^2-hydroxy and
442 15^1-hydroxy lactone derivatives) from digesta of cooked seaweeds (Table 1) with the
443 corresponding AMFs show a clear increment during the micellarization. As it has
444 been established previously (Chen et al., 2018b), oxidized chlorophyll pigments,
445 especially phytylated one, show better micellarization than parent chlorophyll due to
446 the improvement of water solubility by the addition of hydroxyl group. It is
447 noteworthy that the processing does not introduce any modification in the relative
448 content of oxidized chlorophylls in the AMF fraction in red and brown seaweeds
449 (Chen et al., 2018b). In contrast, cooked green seaweeds exhibit significant
450 differences, being the proportion of oxidized chlorophylls even 10% less than in fresh
451 dried AMF. As stated before, the pheophorbidation reactions were strengthened in
452 cooked green seaweeds resulting less substrate for oxidation. An additional effect of
453 the cooking process is the higher content of pyro derivatives (4%) compared with
454 AMF of fresh red seaweeds (1.84%), consequence exclusively to the
455 decarboxymethylation reaction induced during cooking process (Chen et al., 2018c)
456 before digestion.

457 Noteworthy, AMF of cooked Sea Lettuce have less chlorophyll *b* series and
458 13^2-hydroxy derivatives than fresh dried one, which is related with the differences in
459 chlorophyll profile before digestion, as it has been showed earlier more
460 magnesium-containing chlorophylls result more oxidative chlorophyll derivatives
461 (Chen et al., 2018b). Due to the slight decrease of micellarization in *b* series after
462 cooking (Fig. 3), ratios *a*/*b* series increase. On the contrary, ratios of *a*/*c* series in the

AMF of brown seaweed decrease after cooking process and this can be explained by the different decreasing extent of *a* and *c* series (Fig. 3) in micellarization process where *a* series experience a more severe decline in micellarization rate than *c* series.

3.5. Processing bioaccessibility index of chlorophyll pigments in cooked seaweeds

This term is introduced in the purpose of providing information about the exact amounts of chlorophyll pigments that are potentially absorbed in human intestinal taking into account the processing (Fig. S1). This global index has taken in consideration the cooking effects, pigment stability and changes during digestion process, and incorporation ability into micelles. Among species, Nori presents the highest value amounting to 10-12%, followed by Kombu (5-6%) and Sea Lettuce (3-6%) (Fig. 4). Consistently, microwaved seaweeds have higher processing bioaccessibility than boiled seaweeds. It seems samples with microwave introduce fewer interfering compounds into digestive system to impair chlorophyll micellarization process since the decreasing degree is more serious during the boiling of Nori and Kombu seaweeds.

When compared with fresh dried seaweeds (Chen et al., 2018b), the effect of cooking depends on the seaweed specie. For Nori, processing implies a decrease in bioaccessibility index (16% in fresh seaweed), probably due to the lower micellarization rate of chlorophyll pigments in cooked Nori. On the contrary, cooking increases significantly the chlorophyll bioaccessibility in Kombu seaweeds (4% in fresh seaweeds, Chen et al., 2018a) as the recovery rate in cooked Kombu is three times more than in fresh dried Kombu (Fig. 2). Finally, the effect of cooking over the

485 global bioaccessibility of chlorophylls in Sea Lettuce depends of the processing.
486 While boiling affects negatively (from 4% in fresh green seaweeds), microwaving
487 improves the bioaccessibility. For Sea Lettuce, the chlorophyll recovery rate is
488 enhanced by 8% and 37% for boiled and microwaved seaweeds (Chen et al., 2018a).
489 But this effect is compensated by the relatively great loss of chlorophylls during the
490 boiling heating procedure (Chen et al., 2018c).

491 In conclusion, the influences of cooking process on the chlorophyll
492 bioaccessibility are combined consequences of modifications in chlorophyll profile
493 during the seaweed processing, the *in vitro* digestion and following micellarization
494 process. However, the main outcome of this research is the determinant influence of
495 the extracellular matrix among different seaweeds, which balances the evident
496 cooking effects.

497 **Acknowledgements**

498 This work was supported by the Ministerio de Ciencia, Universidades e
499 Innovación, Agencia Estatal de Investigación and European Regional Development
500 Fund (RTI2018-095415-B-I00) and the Chongqing Science and Technology
501 Commission (cstc2018jcyjAX0590) and Southwest University (SWU118101).

502 **Appendix A. Supplementary data**

503 Fig. S1

504 **References**

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FIGURE CAPTIONS

Figure 1: Chlorophyll derivatives and their main reactions described. ① stands for the pheophytinization reaction which replaces the central magnesium with two hydro atoms; ② stands for the pheophorbidation reaction that removes phytyl chain; ③ stands for reactions involved in the fifth isocyclic ring including oxidation reactions to yield 132-hydoxy, 151-hydroxy lactone, purpurin-18 a and chlorin e6 derivatives and decarboxymethylation reaction to yield pyro derivatives. R is CH3 in chlorophyll a and CHO in chlorophyll b.

Figure 2: Recovery of chlorophyll derivatives from cooked seaweeds after in vitro digestion. BN, boiled Nori; MN, microwaved Nori; BS, boiled Sea Lettuce; MS, microwaved Sea Lettuce; BK, boiled Kombu; MK, microwaved Kombu. Total chl. stands for total chlorophylls. Data represent mean ± SEM for 8 independent measurements. Different numbers above the bars means the recovery rate of each series or total content of chlorophyll pigment differs significantly ($p<0.05$) between boiled and microwaved seaweeds of this seaweed.

Figure 3: Micellarization of chlorophyll derivatives from cooked Nori, Sea Lettuce and Kombu samples. Pheo a stands for pheophorbide a type including pheophorbide a, 132-hydroxy pheophorbide a and 151-hydroxy-lactone pheophorbide a; Phy a stands for pheophytin a type including pheophytin a, 132-hydroxy pheophytin a and 151-hydroxy-lactone pheophytin a; Chl b stands for chlorophyll b type including

chlorophyll b, 132-hydroxy chlorophyll b and 151-hydroxy-lactone chlorophyll b; Phy b stands for pheophytin b type including pheophytin b and 132-hydroxy pheophytin b; Chl c stands for chlorophyll c; Pheo c stands for pheophorbide c type including pheophorbide c and oxidized pheophorbide c; Total chl. stands for total chlorophylls. Percentages were calculated by total chlorophyll content in the aqueous micellar fraction (AMF) divided by the amount of chlorophyll pigment at the end of the in vitro digestion process. Data represent mean ± SE for 8 independent measurements. Different numbers above the error bars indicate significant differences of chlorophyll derivatives between boiled and microwaved sample of this seaweed ($p < 0.05$). Different letters above the error bars within the same seaweed indicate significant differences between chlorophyll derivatives ($p < 0.05$).

Figure 4: Processing bioaccessibility index of chlorophyll derivatives from cooked Nori, Sea Lettuce and Kombu seaweeds. Percentages were calculated by total chlorophyll content in the aqueous micellar fraction (AMF) divided by the initial amount of chlorophyll pigment in the fresh dried seaweeds considering the cooking techniques. Data represent mean ± SE for 8 independent measurements. Different numbers above the error bars indicate significant differences of total chlorophylls between boiled and microwaved seaweeds ($p < 0.05$).

Fig S1: Scheme of in vitro digestion and micellarization process

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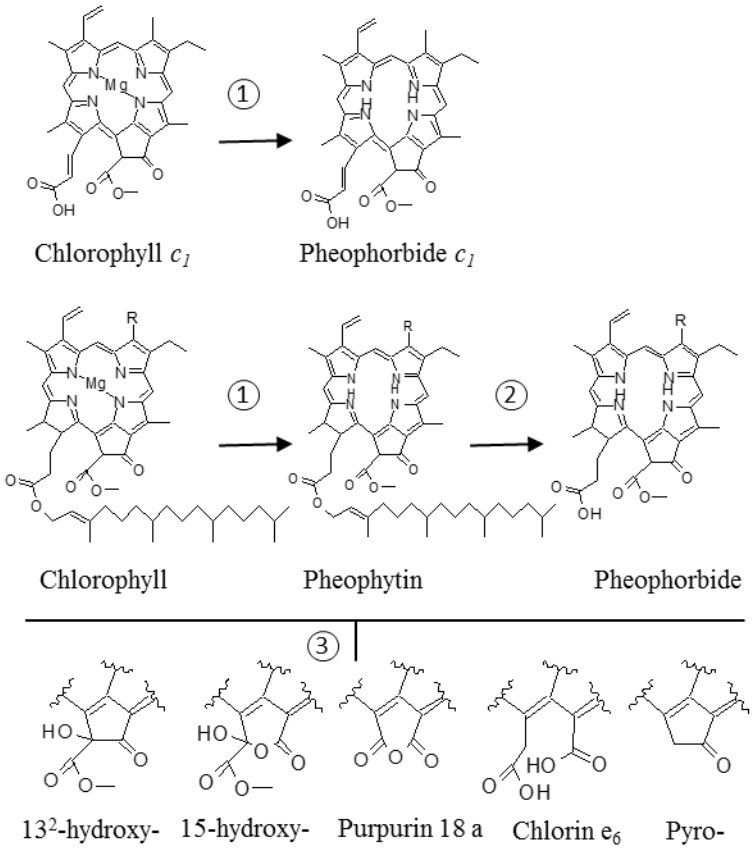
697 Figure 1



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700 Figure 2

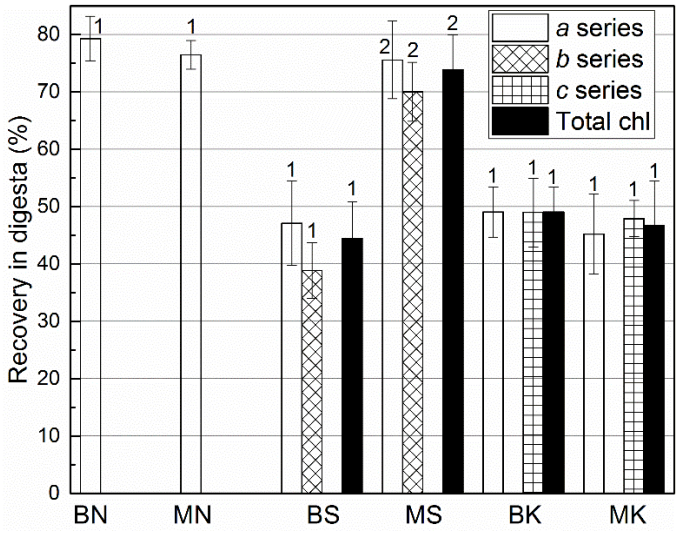

Figure 3

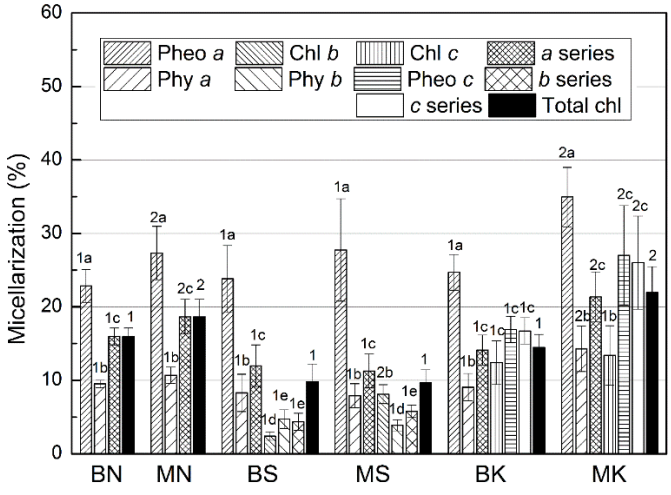

Figure 4

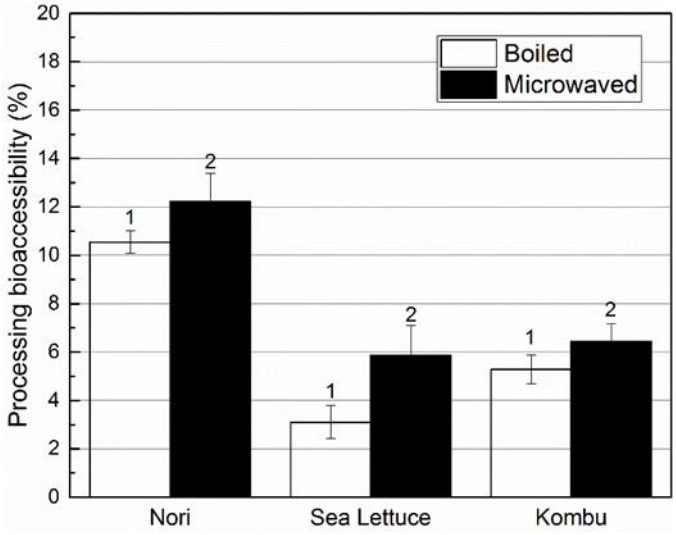

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Table 1: Quantitative distribution (percentage) of individual chlorophyll pigments in cooked Nori, Sea Lettuce and Kombu seaweeds previous (pre-d) and posterior (post-d) to *in vitro* digestion (means ±SE).

	Boiled Nori		Microwaved Nori		Boiled Sea Lettuce		Microwaved Sea Lettuce		Boiled Kombu		Microwaved Kombu	
	pre-d[b]	post-d	pre-d[b]	post-d	pre-d[b]	post-d	pre-d[b]	post-d	pre-d[b]	post-d	pre-d[b]	post-d
Chl *a*									1.86±0.07		2.71±0.42	
15[1]-OH-lact. chl *a*									1.37±0.07		1.74±0.37	
Pheo *a*	32.94±5.48	43.75±1.39	32.80±5.05	43.17±0.82	5.55±0.01	12.62±1.87	6.54±0.17	8.02±0.61	6.22±0.36	25.67±1.46	5.91±0.42	25.51±1.79
13[2]-OH pheo *a*	2.40±0.69	4.28±0.48	2.00±0.30	4.35±0.14	3.01±0.09	3.58±0.54	1.88±0.07	3.42±0.35	0.65±0.18	1.80±0.38	0.33±0.01	3.11±0.64
15[1]-OH-lact. Pheo *a*		0.60±0.30		0.26±0.02	0.60±0.08	1.22±0.29	0.43±0.02	0.77±0.08		0.31±0.06		0.62±0.29
Purpurin-18 *a*	0.35±0.08		0.23±0.02									
Phy *a*	45.63±5.12	34.68±1.39	45.51±3.95	35.22±1.29	40.34±0.06	38.92±3.51	41.28±0.11	40.80±1.43	63.56±1.72	41.82±1.74	63.46±2.16	39.64±2.72
13[2]-OH phy *a*	8.46±0.15	7.53±0.83	8.84±0.71	8.33±1.10	13.18±0.18	12.68±0.59	14.63±0.21	15.45±0.72	8.76±0.57	12.67±0.80	9.47±1.03	12.87±0.54
15[1]-OH-lact. phy *a*	1.90±0.29	2.57±0.37	1.37±0.18	2.07±0.45	3.06±0.07	1.62±0.17	2.59±0.02	1.72±0.10	1.16±0.15	1.44±0.34	1.42±0.15	1.23±0.18
Pyrophy *a*	7.64±0.68	5.92±0.82	8.59±0.79	6.02±0.39	1.84±0.00	1.23±0.39	1.23±0.00	0.75±0.10	1.22±0.15	2.29±0.29	1.71±0.13	2.06±0.34
Phytyl chorin e_6							0.52±0.00					
Phytyl purpurin-18 *a*	0.68±0.17	0.63±0.22	0.66±0.13	0.60±0.11	0.31±0.01		0.21±0.02		1.17±0.15		0.98±0.15	
Chl *b*					11.93±0.02	2.08±0.43	11.27±0.52	2.12±0.08				
13[2]-OH chl *b*					3.18±0.11	0.93±0.24	3.95±0.07	0.82±0.04				
15[1]-OH-lact. chl *b*					0.98±0.02	1.35±0.18	1.20±0.04	1.23±0.14				

Phy *b*					13.42±0.16	16.65±0.68	11.69±0.05	14.98±0.53				
13^2-OH phy *b*					2.59±0.05	7.18±0.54	2.58±0.01	9.97±0.81				
Chl *c*									13.47±1.94	0.49±0.09	11.96±1.65	1.02±0.16
Pheo *c*									0.55±0.20	4.24±0.74	0.31±0.03	4.78±0.89
Oxid. pheo *c*										9.25±0.49		9.16±1.10
Phy *a* series	63.63±6.00	51.33±1.58	64.32±5.28	52.23±0.85	58.42±0.31	54.45±3.74	60.25±0.12	58.72±1.88	74.70±1.45	58.23±1.73	76.06±1.16	55.80±3.16
Phy *b* series					16.01±0.22	23.83±0.83	14.26±0.05	24.95±0.99				
Total phy	**63.63±6.00**	**51.33±1.58**	**64.32±5.28**	**52.23±0.85**	**74.44±0.10**	**78.28±3.26**	**74.51±0.16**	**83.67±1.21**	**74.70±1.45**	**58.23±1.73**	**76.06±1.16**	**55.80±3.16**
Pheo *a* series	35.33±6.15	48.63±1.60	34.79±5.35	47.78±0.83	9.16±0.00	17.42±2.63	8.85±0.27	12.20±1.02	6.87±0.54	27.79±1.66	6.24±0.42	29.24±2.34
Pheo *c* series									0.55±0.20	13.49±1.09	0.31±0.03	13.94±1.94
Total pheo	**35.33±6.15**	**48.63±1.60**	**34.79±5.35**	**47.78±0.83**	**9.16±0.00**	**17.42±2.63**	**8.85±0.27**	**12.20±1.02**	**7.42±0.34**	**41.28±1.75**	**6.55±0.39**	**43.18±3.05**
Chl *a* series									3.24±0.00		4.45±0.04	
Chl *b* series					16.09±0.11	4.37±0.79	16.43±0.41	4.17±0.19				
Chl *c* series									13.47±1.94	0.49±0.09	11.96±1.65	1.02±0.16
Total chl					**16.09±0.11**	**4.37±0.79**	**16.43±0.41**	**4.17±0.19**	**16.70±1.94**	**0.49±0.09**	**16.41±1.69**	**1.02±0.16**
a series					67.89±0.32	71.87±1.45	69.31±0.36	70.92±1.11	85.99±2.14	86.01±1.16	87.73±1.68	85.04±1.99
b series					32.11±0.32	28.20±1.46	30.27±0.34	29.12±1.12				
c series									14.01±2.14	13.99±1.16	12.27±1.6	14.96±1.99

Ratio *a/b*[a]					2.11±0.03	2.56±0.17	2.29±0.04	2.44±0.14				
Ratio *a/c*[a]									6.22±1.10	6.19±0.63	7.23±1.12	5.78±0.85
13^2-OH-deriv.	10.86±0.68	11.82±1.00	10.84±0.43	12.68±1.01	21.96±0.07	24.38±1.31	23.04±0.34	29.65±0.67	9.41±0.38	23.73±0.38	9.80±1.04	25.14±1.00
15^1-OH-lact. series	1.90±0.29	3.17±0.49	1.37±0.18	2.33±0.46	4.65±0.12	4.18±0.58	4.23±0.08	3.72±0.26	2.54±0.21	1.76±0.31	3.16±0.53	1.85±0.27
Pyro deriv.	7.64±0.68	5.92±0.82	8.59±0.79	6.02±0.39	1.84±0.00	1.23±0.39	1.23±0.00	0.75±0.10	1.22±0.15	2.29±0.29	1.71±0.13	2.06±0.34
Purpurin-18 *a* series	1.03±0.15	0.63±0.22	0.89±0.09	0.60±0.11	0.31±0.01		0.21±0.02		1.17±0.15		0.98±0.15	

Abbreviations: Chl, chlorophyll; 15^1-OH-lact., 15^1-hydroxy-lactone; 13^2-OH, 13^2-hydroxy; Pheo, pheophorbide; Phy, pheophytin; oxid., oxidized; deriv., derivatives. [a]Ratio was expressed in numbers; [b]The data of previous digestion (pre-d) come from Chen et al., 2018a.

Table 2: Distribution (percentage) of chlorophyll derivatives in aqueous micellar fractions (AMF) of cooked Nori, Sea Lettuce and Kombu seaweeds (means ±SE; n=8).

	Nori AMF		Sea Lettuce AMF		Kombu AMF	
	Boiled	Microwaved	Boiled	Microwaved	Boiled	Microwaved
Pheophorbide *a*	57,30±1,67	59,29±1,35	30,48±3,09	25.92±1.46	41,33±2,60	41,38±1,12
13^2-hydroxy pheophorbide *a*	10,12±1,20	10,03±0,21	6,95±0,68	10.05±1,84	4,03±0,87	3,51±0,30
15^1-OH-lact. pheophorbide *a*[a]	2,01±0,66	0,93±0,09	4,80±0,67		2,17±0,67	1,80±0,50
Pheophytin *a*	18,48±0,63	17,81±1,02	18,90±1,93	19.18±0,63	20,12±2,14	20,75±1,41
13^2-hydroxy pheophytin *a*	5,68±0,63	4,98±0,32	21,82±4,93	22.48±3,44	13,85±2,42	13,19±1,25
15^1-OH-lact. pheophytin *a*[a]	3,04±1,07	2,52±0,30	4,62±1,15	8.05±3.00	2,24±0,53	1,98±0,21
Pyropheophytin *a*	3,37±0,51	4,44±0,28				
Chlorophyll *b*				1.50±0,37		
13^2-hydroxy chlorophyll *b*			0,57±0,10	0.96±0,33		
15^1-OH-lact. chlorophyll *b*[a]			0,48±0,05	1.22±0.42		
Pheophytin *b*			8,49±1,11	4.38±2,40		
13^2-hydroxy pheophytin *b*			2,89±0,55	6.27±1,05		
Chlorophyll *c*					0,41±0,05	0,62±0,17
Pheophorbide *c*					3,84±0,96	4,93±0,55
Oxidized pheophorbide *c*					12,00±1,22	11,83±0,98
Pheophytin *a* series	30,57±0,71	29,75±1,48	45,34±4,07	49.70±3.03	36,21±3,76	35,92±2,36
Pheophytin *b* series			11,39±1,16	10,65±2,77		
Total pheophytins	**30,57±0,71**	**29,75±1,48**	**56,72±4,07**	**60.35±3.39**	**36,21±3,76**	**35,92±2,36**
Pheophorbide *a* series	69,43±0,71	70,25±1,48	42,22±3,99	35.97±3,06	47,54±2,51	46,70±1,16
Pheophorbide *c* series					15,84±2,13	16,76±1,17
Total pheophorbides	**69,43±0,71**	**70,25±1,48**	**42,22±3,99**	**35.97±3,06**	**63,38±3,79**	**63,47±2,25**
Chlorophyll *b* series			1,05±0,11	3.67±0.68		
Chlorophyll *c* series					0,41±0,05	0,62±0,17
Total chlorophylls			**1,05±0,11**	**3.67±0.68**	**0,41±0,05**	**0,62±0,17**
a series			87,56±1,20	85.67±2.97	83,75±2,11	82,62±1,29
b series			12,44±1,20	14.33±2.97		
c series					16,25±2,11	17,38±1,29
Ratio *a*/*b*[b]			7,11±0,85	6.23±1,46		
Ratio *a*/*c*[b]					5,23±0,74	4.78±0.42
13^2-hydroxy derivatives	15,81±1,43	15,01±0,25	32,23±4,72	39.76±4.56	29,88±1,74	28,53±0,77
Ratio 13^2-OH pheo *a*/pheo*a*[b,c]	0,18±0,03	0,17±0,00	0,23±0,01	0,39±0,06	0,10±0,02	0,09±0,01
Ratio 13^2-OH phy *a*/phy *a*[b,d]	0,31±0,03	0,28±0,02	1,18±0,38	1,17±0,19	0,69±0,12	0,64±0,06
15^1- OH-lactone derivatives	5,05±0,54	3,44±0,31	9,90±0,93	9.26±2.99	4,41±0,95	3,78±0,59
Pyro derivatives	3,37±0,51	4,44±0,28				
	Digesta		Digesta		Digesta	
Ratio 13^2-OH pheo *a*/pheo *a*[b,c]	0.10±0.01	0.10±0.00	0.28±0.01	0.43±0.02	0.07±0.01	0.12±0.02
Ratio 13^2-OH pheo *a*/pheo *a*[b,c]	0.22±0.03	0.24±0.04	0.33±0.03	0.38±0.01	0.30±0.02	0.33±0.02

[a]15^1-OH-lact.: 15^1-hydroxy-lactone; [b]Ratio was expressed in numbers; [c]pheo: pheophorbide; [d]phy: pheophytin. The data of digesta were calculated from Table 1.