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Cooking effects on chlorophyll profile of the main edible seaweeds

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ABSTRACT

Edible seaweeds are rich in chlorophyll pigments, although their modifications during cooking remain unknown. Consequently, the three most consumed seaweeds of different categories: Nori (*Porphyra umbilicales*), Sea Lettuce (*Ulva* sp.) and Kombu (*Laminaria*

ochroleuca) were subjected to two cooking processes, boiling and microwaving. The chemical reactions of the chlorophyll pigments were determined by HPLC-UV/Vis. Besidesthe main chlorophyll transformations already described in fruits and vegetables (pheophytinisation and decarboxymethylation), the cooking of seaweeds caused a high level of oxidative reactions. Statistically, Nori was the most resistant algae to heating, while Sea Lettuce was the most labile seaweed. We report the thermal stability of *c* series for the first time, which were significantly less stable than *a* series in Kombu . Differences after microwaving and boiling methods depend mainly on the seaweed class. In conclusion, the seaweed structure is the main factor that determines the influence of cooking on the chlorophyll profile.

Keywords: chlorophylls; cooking; Kombu; Nori; oxidation; pheophytin; Sea Lettuce; seaweeds.

1. Introduction

Edible seaweeds, or marine macroalgae, have been consumed in Asia as part of traditional cuisine since ancient times, but in European countries and the US, seaweeds are generally consumed for their nutritional value (Buschmann *et al*., 2017; Rioux, Beaulieu, & Turgeon, 2017). The Food and Agriculture Organization (FAO, 2016) estimated that annual seaweed production was over 30 million tonnes of fresh weight, and predicted an annual growth rate of 6%. Moreover, the European Commission (2016) recognised that by 2054, algal collective cultivation could produce 56 million metric tonnes of protein, accounting for 18% of all global protein production (Buschmann *et al*., 2017). In addition to their nutritional value, edible seaweeds are also a good source of bioactive compounds (Gupta & Abughannam, 2011; Pangestuti & Kim, 2011).

Chlorophyll pigments are one group of bioactive compounds present in edible seaweeds and have been proven to harbour important biological properties, such as antioxidant, antimutagenic

and anti-inflammatory effects (Lanfer-Marquez, Barros, & Sinnecker, 2005; Subramoniam *et al*., 2012). Moreover, the pigments are bioavailable and distributed in the serum (Egner, Stansbury, Snyder, Rogers, Hintz, & Kensler, 2000). In fact, seaweed chlorophylls have been shown to be resistant to *in vitro* digestion and are potentially absorbed by the intestinal epithelium (Chen & Roca, 2018a, b).

Different categories of seaweeds contain a different chlorophyll profile (Chen, Ríos, Pérez-Gálvez, & Roca, 2017). The phyla Rhodophyta and Phaeophyta mainly contain magnesium detached derivatives, including pheophorbides and pheophytins; whereas, phylum Chlorophyta mainly contain magnesium-containing chlorophyll derivatives (Hwang *et al*., 2005; El-Baky, El-Baz, & El-Baroty, 2009; Amorim, Lage-Yusty, & López-Hernández, 2012). In addition, diverse oxidized chlorophyll derivatives form part of the photosynthetic tissues in edible seaweeds, including $C13^{2}$-hydroxy and $C15^{1}$-hydroxy lactone chlorophyll derivatives and opening structures (Chen *et al.*, 2017). The presence of diverse chlorophyll compounds means that different chlorophyll structures exhibit distinct functional properties (Lanfer-Marquez, Barros, & Sinnecker, 2005) and bioavailability characteristics (Chen & Roca, 2018a, b). To date, no studies have investigated the influence of different cooking methods on the chlorophyll pigments of edible seaweeds. Thermal investigations of chlorophylls in food have been exclusively carried out in green fruits and vegetables (Schwartz & Lorenzo, 1991; Van Loey *et al*., 1999; Turkmen, Poyrazoglu, Sari, & Velioglu, 2006; Koca, Karadeniz, & Burdurlu, 2007), which contain chlorophyll *a*, chlorophyll *b* and small amounts of pheophytin *a.* In these investigations, the main transformations to chlorophyll pigments associated with cooking are pheophytinisation and decarboxymethylation reactions.

Besides their more complex chlorophyll profile, edible seaweeds contain a higher amount of fibre than vegetables, up to 40% (Rioux, Beaulieu, & Turgeon, 2017), as well as a complex of various polysaccharides, such as agars and carrageenans in red algae, and fucoidans and alginates in brown algae (Synytsya, Čopíková, Kim, & Yong, 2015). Anatomical studies have revealed that thermal treatment can modify the structure of the extracellular matrix (Zaupa, Ganino, Dramis, & Pellegrini, 2016), while the effects of the cooking process on the chlorophyll pigments remain unknown.

Therefore, the aim of this study was to determine the effects of different cooking methods on the chlorophyll profile of the main edible seaweeds and define whether the seaweed species, cooking method or chlorophyll structure is the key factor influencing the chlorophyll profile of seaweeds.

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

Sodium chloride, ammonium acetate (98%) and tetrabutylammonium acetate were supplied by Sigma-Aldrich Chemical Co. (Madrid, Spain). Acetone (analysis grade) was supplied by

Teknokroma (Barcelona, Spain). *N*,*N*-dimethylformamide grade solvents and water were supplied by Panreac (Barcelona, Spain), while acetone HPLC grade was supplied by Merck (Madrid, Spain). The deionised water used was obtained from a Milli-Q 50 system (Millipore Corp., Milford, MA, USA).

2.3. Cooking treatments

Fresh dried seaweeds (Nori, Sea Lettuce and Kombu) were cut into sizes of about 1×1cm squares, divided into three equal portions (15 g) and combined with water in a ratio of 1:20 (w/v). One portion of seaweed sample was retained raw; the other two were subjected to heating procedure, being both beakers with the mixtures of seaweeds and water covered with a plate. The boiling was conducted at 100 ºC for 20 min, while the microwaving (microwave oven Taurus, model Speedy 21L, Barcelona, Spain) was developed with a output power of 800 W for 15 min). Cooking conditions were according to the consumer habits and product instructions provided by seaweed suppliers. After that, samples were lyophilized for pigment extraction.

2.4. Pigment extraction

Following the method previously detailed (Chen *et al*., 2017), the seaweeds were mixed with liquid nitrogen and grinded into powder in a mortar. The particle size of obtained powder was reduced by passing through 576 meshes/cm^2 sieves, equivalent to a particle size of 0.5 mm (Φ). Then 0.2 grams of seaweed powder were mixed with 30 mL of extraction solvent, *N*,*N*-dimethylformamide:water (9:1). After extraction and filtration, samples were subjected to HPLC analysis. Samples were stored at -20^{o}C until analysis and moisture content of obtained seaweeds powder was determined by standard moisture analyser (OHAUS, MB35).

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

The pigments were separated by reversed-phase HPLC using a Hewlett-Packard HP 1100

liquid chromatograph. A Mediterranea Sea18 column (200×4.6 mm, 3 μm particle size) was used (Teknokroma, Barcelona, Spain) protected by a guard column (10×4.6 mm) packed with the same material. Separation was performed using the elution gradient described by Chen *et al.* (2017). The on-line UV-Visible spectra were recorded from 350 to 800 nm with the photodiode–array detector and sequential detection was performed at 410, 430, 450 and 666 nm. Data were collected and processed with a LC HP ChemStation (Rev.A.05.04). Identification of chlorophyll derivatives was made based on co-chromatography with authentic samples and from their spectral characteristics (Chen, & Roca, 2018a, b), except for chlorin e_6 and oxidized pheophorbide c_1 whose identification is tentative only based in spectral and polarity characteristics (Mínguez-Mosquera, & Gandul-Rojas, 1995; Garrido, Otero, Maestro, & Zapata, 2000). Quantification of pigments was performed with the corresponding calibration curves (amount versus integrated peak area). The calibration equations were obtained by least-squares linear regression analysis over a concentration range according to the observed levels of these pigments in the analysed samples. Injections in duplicate were made for five different volumes at each standard solution.

2.6. Statistical analysis

A triplicate was made for each analysis. One-way analysis of variance (ANOVA, StatSoft, Inc., 2001) was performed to evaluate the effect of the treatment on each type of algae.

3. Results and discussion

3.1. Modifications of the chlorophyll profile during the cooking process

The full mass characterisation of chlorophyll derivatives in the fresh main edible seaweeds has been recently studied (Chen *et al.*, 2017) and the chlorophyll distribution has been described (Hwang *et al.*, 2005; El-Baky, El-Baz, & E-Baroty, 2009). In the present study, we identified all

the chlorophyll structures found during the cooking process of Nori, Sea Lettuce and Kombu, including *a*, *b* and *c* series chlorophyll (Figure 1). Tables 1 and 2 show the variation of chlorophyll derivatives in commercialised fresh dried alga and after the cooking procedures of boiling and microwave heating, both of which exerted modifications to the chlorophyll profile.

For the red algae Nori (Table 1), the amount of total chlorophyll pigments was only slightly reduced by the boiling process and microwave treatment. The main modifications introduced by both cooking treatments were the complete degradation of chlorophyll *a* and the formation of pyropheophytin *a*. In addition, boiling but not microwaving caused a net increase of 15^1-OH-lactone pheophytin *a* and purpurin-18 *a*. The fresh dried starting materials were mainly composed of pheophorbides and pheophytins of series *a* (>97%), and the cooking process did not modify such a profile.

In the case of the green algae, Sea Lettuce, cooking caused the net degradation of total chlorophyll pigments, with a loss of 28% and 14% after the boiling and microwave processes, respectively (Table 1). This degradation affected series *a* chlorophyll (35% and 21% for boiling and microwave, respectively) more than series *b* (less than 10% for boiling and unchanged in net *b* series content after microwaving), with cooking resulting in a decrease of the *a*/*b* series ratio. Specifically, both cooking processes caused the complete transformation of chlorophyll *a* and 13^2-OH-chlorophyll *a* in favour of pheophytin *a* and its derivatives. A parallel reaction, albeit to a lesser extent, also occurred for series *b* chlorophylls. The pheophytinisation reaction affected over 50% of chlorophyll *b* after Sea Lettuce cooking. As a result, the main effect caused by the cooking of Sea Lettuce was a 40% increase of pheophytin *a* and a 15% increase of pheophytin *b* compared to dry weight algae (Table 2). A second consequence of the cooking process was an increase in oxidation reactions, with significant increments of 15^1-OH-lactone and 13^2-OH

chlorophyll derivatives. For *a* series pigments, the oxidative reaction mainly affected pheophytin compounds, while for the *b* series, oxidation affected both chlorophyll and pheophytin structures. Curiously, pheophorbide *a* seemed more resistant to oxidation. In addition, cooking induced the formation of pyropheophytin *a*, but not pyropheophytin *b*, in parallel to the increase of phytyl-purpurin-18 *a*. The formation of phytylchlorin e_6 was tentatively identified only for microwaved Sea Lettuce. As a consequence of all the reactions that occur during the cooking process, the chlorophyll profile of Sea Lettuce after cooking is completely different to the profile for the fresh dried form. Fresh green seaweeds are dominated by chlorophylls (73%), while cooked Sea Lettuces are dominated by pheophytins (74%), with minor amounts of chlorophylls (16%) and pheophorbides (9%). In addition, oxidative chlorophylls represent more than 25% of the chlorophyll profile in cooked green seaweeds.

For the brown algae, Kombu, the cooking process caused a loss of around 30% of the total chlorophyll pigments, an even higher loss than for green algae; however, for the pheophyceae seaweed, the chlorophyll degradation occurred at the same level after boiling and microwaving. The net degradation of chlorophyll affected the *c* series (37% and 53% for boiling and microwave, respectively) more than the *a* series of pigments (23% and 33% for boiling and microwave, respectively), and as a result, the *a/c* ratio was higher for cooked seaweeds than for fresh ones (Table 2). For series *a*, a net degradation of chlorophyll *a* can be observed without an associated increase of the corresponding pheophytin *a*. Cooking also caused the pheophytin *a* degradation of brown seaweeds. Although the total starting amount of chlorophyll *a* in Kombu (225 mg/kg) was much less than in green seaweeds (4319 mg/kg in fresh dried material, Table 1), the degradation of chlorophyll *a* in the brown seaweed was not complete, and cooked Kombu still contained some chlorophyll *a* in its profile unlike the other edible seaweeds tested. For the *c*

series pigments, pheophorbide c_1 was formed after the cooking of brown seaweed from chlorophyll c_1, but the transformed amounts did not account for the total loss of chlorophyll c_1, implying a net degradation of chlorophyll c_1. Interestingly, the cooking of brown seaweeds did not cause an increase of the total oxidized chlorophylls or the 13^2-OH or 15^1-OH-lactone-derivatives. On the contrary, it caused the significant degradation of these compounds. In Kombu, and similar to the results observed in Sea Lettuce, boiling and microwaving caused a net increase of pyropheophytin *a* (but not pyropheophorbide *c*) and phytyl-purpurin-18 *a*. In summary, the cooking process transformed the chlorophyll profile of the fresh brown seaweed due to the greater degradation of chlorophylls than pheophytins. The cooking of Kombu increased the relative abundance of pheophytins (75%) and pheophorbides (7%) at the detriment of chlorophylls (16%).

3.2. Comparison of chlorophyll modifications during the cooking process of different seaweed classes

Chlorophyll degradation in vegetables after processing has been widely studied (Schwartz & Lorenzo, 1991; Van Loey *et al.*, 1998; Teng & Chen, 1999; Turkmen, Poyrazoglu, Sari, & Velioglu, 2006; Koca, Karadeniz, & Burdurlu, 2007), with the conclusion that the net loss of chlorophylls depends not only on the cooking method employed but also on the type of vegetable. This is the first time the effects of cooking on the chlorophyll profile of edible seaweeds have been reported. The differences we report in the net degradation of chlorophylls among Nori, Sea Lettuce and Kombu are reasonable, taking into account the additional complex extracellular composition of the different phyla of macroalgae (Synytsya, Čopíková, Kim, & Yong, 2015). Such differences also affect other compounds in seaweeds (Almela, Laparra, Vélez, Barberá, Farré, & Montoro, 2005) during the cooking process.

Pheophytinisation is the main reaction that affects chlorophyll *a* and *b* during cooking (Teng & Chen, 1990) and is associated with the amount of acids liberated during processing (Schwartz & Lorenzo, 1991). Analysis of the pheophytinisation reaction revealed that the exchange of magnesium for hydrogen is energetically advantageous (Rutkowska-Zbik & Korona, 2012) and that pheophytins have much higher thermal stability than chlorophylls. In this study, the behaviour of the three seaweeds was surprising in this aspect: In Sea Lettuce, with the highest starting amount of chlorophyll, the chlorophyll *a* fractions were completely transformed by cooking. This was also true for Nori, although with lower starting amounts. In contrast, 25% of chlorophyll *a* remained intact after the cooking of Kombu, with around 5% of the starting levels of total chlorophyll *a* of Sea Lettuce in its fresh dried form . We hypothesise that the extent of the pheophytinisation reaction is determined by the distinct cellular structure and extracellular composition of the different classes of seaweeds, either facilitating (in the case of Sea Lettuce) or complicating (in the case of Kombu) the liberation of acids.

The cooking of green seaweed allowed us to compare the effects of cooking on the pheophytinisation of series *a* and *b* pigments, with the *b* series demonstrating higher stability. Chlorophyll *a* and chlorophyll *b* were transformed into pheophytin *a* and *b* following first order kinetics upon heating (Schwartz & Elbe, 2010; Weemaes, Ooms, Van Loey, & Hendrickx, 1999), with the subsequent degradation of magnesium-free chlorophyll derivatives (pheophorbide and pheophytin) requiring a higher activation energy than green chlorophylls (Weemaes, Ooms, Van Loey, & Hendrickx, 1999). The kinetics of the pheophytinisation of both series have been analysed in depth and the energy of activation determined, and, in general, chlorophyll *b* is more resistant than chlorophyll *a* (Gaur, Shivhare, Sarkar, & Ahmed, 2007; Gaur-Rudra, Sarkar, & Shivhare, 2008). The results obtained for green seaweeds suggest that once an acidic

environment surrounds the chlorophyll compounds, the structural change of the chlorophyll molecule affects the overall structure of the macroalgae, similar to the reaction that occurs in other cooked vegetables. In parallel, the cooking of brown seaweed allowed us to analyse the stability of chlorophyll *c*, showing that the amount of pheophorbide *c* formed during the cooking process is very low. For the first time these data suggest that the substitution of magnesium by hydrogen in chlorophyll molecules is more difficult for chlorophyll *c* than for chlorophyll *a*. As chlorophyll *c* is only present in certain marine algae, our study is the first to demonstrate the resistance of chlorophyll *c* to the replacement of its central magnesium, a result in accordance with the recently reported high digestive stability of chlorophyll *c* (Chen & Roca, 2018a). The reaction of pheophytinisation may be less favoured in chlorophyll c_1 because of its structure (structure C, Fig. 1). This chlorophyll derivative is more stable than other series of chlorophyll compounds due to its lower π-electron density in the planar porphyrin ring caused by the extra double bond between $C17^1$ and $C17^2$ in combination with a different unsaturation arrangement in the porphyrin macrocycle (Sadaoka *et al*., 2013).

It has long been shown that the application of high temperature to foods induces the decarboxymethylation of chlorophylls at $C13^2$ originating pyroderivatives (Teng & Chen, 1999; Schwartz & Elbe, 2010). Interestingly, this reaction exclusively affects pheophytin compounds in seaweeds but not chlorophylls or pheophorbides (Tables 1 and 2) due to two other functional groups: the central magnesium and the phytyl chain. Both the replacement of magnesium and the decarboxymethylation reaction can occur at high temperatures, although the detachment of magnesium is more common as it can also be induced by the organic acid released from plant tissue. As a result, the formation of pyropheophytins is frequently found after the heating of green tissues (Schwartz & Elbe, 2010), while the formation of pyrochlorophyll is rare (Teng &

Chen, 1999). Furthermore, it has been showed that the phytyl chain not only causes steric hindrance for chlorophyll reactions but also interacts with some peripheral groups of the isocyclic ring of the macrocycle (Fiedor, Kania, Myśliwa-Kurdziel, Orzeł, & Stochel, 2008). The removal of the phytyl chain (as in pheophorbide) may result in an increased macrocycle flexibility and the steric configuration of the carboxymethyl group, thus making the decarboxymethylation of dephytylated derivatives more difficult, as seen in our analysis. In addition, only pyropheohytin *a* was formed, not pyropheophytin *b* or pyropheophorbide c_1. Compared with *a* series pigments, *b* and *c* series pigments have an additional unsaturated site in their molecule, which shows an electron withdraw effect from the macrocycle, thus making the molecule less susceptible to electrophilic attack (Orzeł, Kania, Rutkowska-Zbik, Susz, Stochel, & Fiedor, 2010). However, a more important factor on the reaction viability than the chlorophyll configuration is the structure of the algae, which determines the extent of the reaction. In terms of the pheophytinisation reaction, the rigidity of the extracellular material of the seaweeds probably protects the pigments from the influence of heat to different extents. For example, the decarboxymethylation reaction occurs to a greater extent in Sea Lettuce, followed by Kombu and Nori.

The allomerisation of chlorophylls is not a reaction generally associated with the cooking of vegetables; however, in the case of seaweeds, cooking induces the allomerisation reaction, and, at least for the green seaweeds, to a high extent. Studies of the thermodynamics of oxidized chlorophylls (Aparicio-Ruiz, Mínguez-Mosquera, & Gandul-Rojas, 2010) have shown that the matrix is a determinant factor in their formation. In addition, the presence of antioxidants in the matrix could interfere in the free radical mechanism responsible for chlorophyll oxidation. In this sense, the different extent of oxidation reactions that occurred in the three edible seaweeds

studied can be explained in terms of accessibility to the pigment as well as the distinct contents of antioxidants in the three species. It is noteworthy that chlorophyll c_1 and pheophorbide c_1 are neither prone to oxidation at $C13^2$ or 15^1, nor decarboxymethylation, nor opening at the fifth isocyclic ring by the cooking process. As previously established, the structural configuration of the chlorophyll *c* series means these molecules are more stable than other chlorophyll series (Sadaoka *et al*., 2013). In addition, purpurin-18 *a* and its phytyl derivative represent an additional level of oxidation at the E ring (Fig. 1) and have been classified as catabolites during the oxidation of chlorophylls in fresh dried seaweeds and in sediments (Chen *et al.,* 2017). We found that levels of phytylpurpurin-18 *a* only increased during the cooking of Sea Lettuce and Kombu seaweeds but not in Nori. Phytylchlorin e_6 is a highly oxidised chlorophyll compound with an open isocyclic ring (Fig.1 structure E). Although this compound has piqued interest due to its properties as a photosensitiser in photodynamic therapy (Pérez-Gálvez & Roca, 2017), only the derivative 15^2-methyl-phytyl chlorin e_6 ester has been associated with food processing (Aparicio-Ruiz, Riedl, & Schwartz, 2011), specifically during the alkaline treatment of olive fruits. Its formation exclusively in Sea Lettuce confirms the higher susceptibility of this seaweed to pro-oxidation reactions.

Comparisons of the stability of *a* and *b* series pigments to high temperature treatments (in terms of resistance to degradation) consistently show that *a* series molecules are less stable than *b* series molecules (Van Loey *et al*., 1998; Koca, Karadeniz, & Burdurlu, 2007), a property attributed to the electron-withdrawing effect of the C3 formyl group, and show greater degradation at high temperature, in accordance with our results for Sea Lettuce. For chlorophyll c_1, only a higher stability than chlorophyll *a* in relation to photochemical bleaching has been determined (Jeffrey & Allen, 1964). To the best of our knowledge, this is the first time the

thermal stability of *c* series pigments has been analysed, and we show that the chlorophyll *c* series are more prone to thermal degradation in comparison with series *a* molecules.

3.3. Comparison of chlorophyll modifications in edible seaweeds according to cooking style

Thermal processing of food helps to improve the food's palatability, inactivate pathogens and microbial hazards, and enhance the digestibility of food and the bioavailability of nutrients (LingTang, Kong, Mitcham, & Wang, 2015). Microwaves, lying between infrared radiation and radio waves in the electromagnetic spectrum, are a convenient and effective tool for heating procedures both in the home and in industry. In terms of the energy transfer to polar molecules in food, microwave heating achieves a non-contact and rapid heating effect from the interior to the exterior of the food, resulting in a totally different temperature gradient compared with traditional cooking methods such as boiling, which is conducted by heat transfer from the opposite direction (Navarro, Flores, & Stortz, 2007).

To the best of our knowledge, no research has been conducted to compare the effects of microwaving or boiling of seaweeds. Other independent investigations have evaluated the effects of microwaving or boiling for the extraction of functional compounds (mainly polysaccharides), finding that they enhance or modify certain molecular properties. Studies related to chlorophyll pigments have exclusively evaluated heating effects in fruits and vegetables (Van Loey *et al*., 1998; Turkmen, Poyrazoglu, Sari, & Velioglu, 2006; Koca, Karadeniz, & Burdurlu, 2007), establishing that both heating processes depress the enzymatic reactions associated with chlorophyll catabolism due to the high temperatures induced. In conclusion, it was determined that chlorophyll transformation or degradation mainly depend on the chlorophyll molecule's chemical properties. In our study with seaweeds, significant differences between cooking methods were found in Sea Lettuce and to a lesser extent in Kombu (Table 2). Possibly the

different extracellular composition of the three seaweeds determines the impact of the cooking process. In fact, it has been shown that the consequences of cooking on other phytochemicals is affected by the seaweed structure, with higher phytochemical contents maintained in more rigid seaweed structures (Amorim, Lage-Yusty, & López-Hernández, 2012). In Sea Lettuce, the net degradation of chlorophylls was lower for microwaved seaweeds than boiled ones. Accordingly, it has been proven that microwave heating can help to retard the colour loss in some food matrices that contain phytochemical compounds (Benlloch-Tinoco, Kaulmann, Cortereal, Rodrigo, Martínez-Navarrete, & Bohn, 2015).

4. Conclusion

Cooking causes modifications to the structure and composition of foods. Due to the complexity and differences of extracellular material among macroalgae classes, seaweeds are an interesting food matrix to study in terms of analysis of the consequences of the cooking process. In general, chlorophylls are considered to be labile phytochemicals with high reactivity caused by modifications to pH and temperature. Surprisingly, we revealed that the effect of cooking on the chlorophyll profile of the main edible seaweeds depended mainly on the seaweed's category. Nori stood out as the most resistant seaweed to both boiling and microwave heating in terms of the stability of chlorophyll pigments, while the green seaweed Sea Lettuce experienced a higher level of chlorophyll transformations. In addition, the rich profile of chlorophyll compounds present in seaweeds allowed us to analyse the thermal stability of chlorophyll *c* series for the first time. Our study in seaweeds showed that cooking induces pheophytinisation and decarboxymethylation, in common with other food matrices such as fruits and vegetables. However, one particularly unusual reaction described after the cooking of seaweeds was chlorophyll oxidation, which even opened the isocyclic ring in some cases. The nutritional and

healthy properties of each chlorophyll derivative are different as the chemical structure determines its biological function. Consequently, modifications in the chlorophyll profile of the different seaweeds as a consequence of the cooking process imply variations in the nutritional value of each foodstuff. Finally, the class of seaweed determined the influence of different cooking methods probably due to the different mechanism of energy transfer caused by microwaving or boiling. **Conflict of interest**

The authors declare that there are no conflicts of interest.

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Figure caption

Figure 1: Structures and names of chlorophyll derivatives from *a*, *b* and *c* series of chlorophyll pigments found during the cooking process of Nori, Sea Lettuce and Kombu seaweeds.

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Table 1: Quantification of chlorophyll derivatives in Nori, Sea Lettuce and Kombu edible seaweeds with different cooking procedures (mg/kg d.w. ±SD)

Table 2: Percentages and ratios of chlorophyll derivatives in Nori, Sea Lettuce and Kombu edible seaweeds before and after cooking.

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Table 1: Quantification of chlorophyll derivatives in Nori, Sea Lettuce and Kombu edible seaweeds with different cooking procedures (mg/kg dry weight±SD)

	Nori			Sea Lettuce			Kombu		
	Fresh dried	Boiled	Microwaved	Fresh dried	Boiled	Microwaved	Fresh dried	Boiled	Microwaved
Chlorophyll *a*	46.39±6.92			4319±124			225±3	43.57±4.95	54.63±3.23
13^2-hydroxy chlorophyll *a*				511±19					
15^1-OH-lac. chlorophyll *a*[a]							75.44±6.63	32.04±0.85	34.50±4.39
Pheophorbide *a*	926±89	720±50	729±49	454±7	396±0	556±29	144±8	145±3	118±2
13^2-hydroxy pheophorbide *a*	41.67±8.23	53.01±7.43	44.41±5.91	209±7	215±7	160±10	13.59±4.66	15.07±3.15	6.53±0.72
15^1-OH-lac. pheophorbide *a*[a]				28.95±1.59	43.00±5.46	36.7±2.83			
Purpurin-18 *a*	4.79±0.55	7.37±1.11	5.07±0.53						
Pheophytin *a*	1156±25	974±29	1010±102	1129±44	2882±1	3509±79	1719±74	1483±73	1269±70
13^2-hydroxy pheophytin *a*	211±20	182±24	197±22	660±28	941±11	1244±49	369±8	205±29	191±38
15^1-OH-lac. pheophytin *a*[a]	32.19±4.71	40.51±1.18	30.35±3.26	116±21	219±5	220±7	37.09±1.30	27.06±1.34	28.38±0.54
Pyropheophytin *a*	150±9	164±12	191±19		131±0	104±3	9.96±0.68	28.26±1.30	34.27±5.68
Phytyl chlorin						43.88±0.81			
Phytyl purpurin-18 *a*	14.55±1.33	14.51±1.37	14.54±2.17	14.53±0.13	21.89±0.91	18.01±1.01	10.53±1.98	27.24±1.40	19.56±1.24
Chlorophyll *b*				2357±147	852±1	958±20			
13^2-hydroxy chlorophyll *b*				122±25	227±8	336±14			
15^1-OH-lac. chlorophyll *b*[a]				30.17±4.78	70.30±1.63	102±6			
Pheophytin *b*					959±13	994±30			
13^2-hydroxy pheophytin *b*					185±4	219±5			
Chlorophyll c_1							522±51	316±39	241±24
Pheophorbide c_1								12.90±2.56	6.23±1.13
Total pheophytins	**1550±28**	**1361±56**	**1428±139**	**1905±57**	**5317±2**	**6291±173**	**2136±84**	**1743±99**	**1522±113**
Total pheophorbides	**968±163**	**773±242**	**774±137**	**692±15**	**655±1**	**753±42**	**158±16**	**173±5**	**131±4**
Total chlorophylls	**46.39±6.92**	**0**	**0**	**7339±290**	**1150±10**	**1396±0**	**823±61**	**392±75**	**330±63**
Total chl pigments[b]	**2584±151**	**2156±287**	**2221±158**	**9950±362**	**7144±12**	**8502±214**	**3127±134**	**2335±178**	**2002±179**

[a] 15^1-OH-lac.: 15^1-hydroxy-lactone; [b] total chl pigments, total chlorophyll pigments.

Table 2: Percentages and ratios of chlorophyll derivatives in Nori, Sea Lettuce and Kombu edible seaweeds before and after cooking.

	Nori			Sea Lettuce			Kombu		
	Fresh dried	Boiled	Microwaved	Fresh dried	Boiled	Microwaved	Fresh dried	Boiled	Microwaved
Pheophytin *a* series (%)	60.14±3.94	63.63±6.00	64.32±5.28	19.15±0.12	58.42±0.31	60.25±0.12	68.29±0.23	74.70±1.45	76.06±1.16
Pheophytin *b* series (%)					16.01±0.22	14.26±0.05			
Total pheophytins (%)	**60.14±3.94**	**63.63±6.00**	**64.32±5.28**	**19.15±0.12**	**74.44±0.10**	**74.51±0.16**	**68.29±0.23**	**74.70±1.45**	**76.06±1.16**
Pheophorbide *a* series (%)	37.31±4.27	35.33±6.15	34.79±5.35	6.96±0.10	9.16±0.00	8.85±0.27	5.07±0.63	6.87±0.54	6.24±0.42
Pheophorbide *c* series (%)								0.55±0.20	0.31±0.03
Total pheophorbides (%)	**37.31±4.27**	**35.33±6.15**	**34.79±5.35**	**6.96±0.10**	**9.16±0.00**	**8.85±0.27**	**5.07±0.63**	**7.42±0.34**	**6.55±0.39**
Chlorophyll *a* series (%)	1.81±0.33			48.55±0.71			9.62±0.11	3.24±0.00	4.45±0.04
Chlorophyll *b* series (%)				25.20±0.94	16.09±0.11	16.43±0.41			
Chlorophyll *c* series (%)							16.68±0.92	13.47±1.94	11.96±1.65
Total chlorophylls (%)	**1.81±0.33**			**73.75±0.23**	**16.09±0.11**	**16.43±0.41**	**26.31±0.81**	**16.70±1.94**	**16.41±1.69**
a series (%)	100	100	100	74.80±0.94	67.89±0.32	69.31±0.36	83.32±0.92	85.99±2.14	87.73±1.68
b series (%)				25.20±0.94	32.11±0.32	30.27±0.34			
c series (%)							16.68±0.92	14.01±2.14	12.27±1.68
Ratio *a/b*				2.97±0.15	2.11±0.03	2.29±0.04			
Ratio *a/c*							5.00±0.33	6.22±1.10	7.23±1.12
13^2-OH derivatives (%)	9.84±1.12	10.86±0.68	10.84±0.43	15.11±0.70	21.96±0.07	23.04±0.34	12.24±0.42	9.41±0.38	9.80±1.04
15^1-OH lactone series (%)	1.24±0.11	1.90±0.29	1.37±0.18	1.75±0.49	4.65±0.12	4.23±0.08	3.60±0.10	2.54±0.21	3.16±0.53
Pyro derivatives (%)	5.81±0.37	7.64±0.68	8.59±0.79		1.84±0.00	1.23±0.00	0.32±0.01	1.22±0.15	1.71±0.13
Purpurin series (%)	0.75±0.03	1.03±0.15	0.89±0.09	0.15±0.00	0.31±0.01	0.21±0.02	0.34±0.05	1.17±0.15	0.98±0.15

All the numbers except ratios between *a* and *b* or *c* series were expressed in percentage of respective chlorophyll derivatives to total chlorophyll content.

Figure 1

Compound	Structure	R_1	R_2	R_3	R_4	R_5
		Series *a*				
Chlorophyll *a*	A	CH_3	H	$COOCH_3$	phytyl	Mg
13^2-hydroxy chlorophyll *a*	A	CH_3	OH	$COOCH_3$	phytyl	Mg
15^1-hydroxy-lactone chlorophyll *a*	B	CH_3	OH	$(15^2)COOCH_3$	phytyl	Mg
Pheophorbide *a*	A	CH_3	H	$COOCH_3$	H	2H
13^2-hydroxy pheophorbide *a*	A	CH_3	OH	$COOCH_3$	H	2H
15^1-hydroxy-lactone pheophorbide *a*	B	CH_3	OH	$(15^2)COOCH_3$	H	2H
Purpurin-18 *a*	D	CH_3	-	-	H	2H
Pheophytin *a*	A	CH_3	H	$COOCH_3$	phytyl	2H
13^2-hydroxy pheophytin *a*	A	CH_3	OH	$COOCH_3$	phytyl	2H
15^1-hydroxy-lactone pheophytin *a*	B	CH_3	OH	$(15^2)COOCH_3$	phytyl	2H
Pyropheophytin *a*	A	CH_3	H	H	phytyl	2H
Phytyl purpurin-18 *a*	D	CH_3	-	-	phytyl	2H
Phytyl chorin e_6	E	CH_3	-	-	phytyl	2H
		Series *b*				
Chlorophyll *b*	A	CHO	H	$COOCH_3$	phytyl	Mg
13^2-hydroxy chlorophyll *b*	A	CHO	OH	$COOCH_3$	phytyl	Mg
15^1-hydroxy-lactone chlorophyll *b*	B	CHO	OH	$(15^2)COOCH_3$	phytyl	Mg
Pheophytin *b*	A	CHO	H	$COOCH_3$	phytyl	2H
13^2-hydroxy pheophytin *b*	A	CHO	OH	$COOCH_3$	phytyl	2H
		Series *c*				
Chlorophyll c_1	C	CH_3	H	$COOCH_3$	H	Mg
Pheophorbide c_1	C	CH_3	H	$COOCH_3$	H	2H