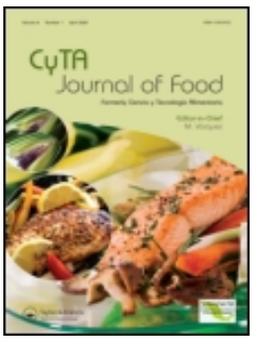

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SHORT COMMUNICATION

Variation of bioactive compounds in dried seaweed *Himanthalia elongata* subjected to different culinary processes

Variación de compuestos bioactivos en algas marinas *Himanthalia elongata* deshidratadas expuestas a distintos procesos culinarios

Kamila Amorim-Carrilho, Maria Asunción Lage-Yusty and Julia López-Hernández*

Department of Analytical Chemistry, Nutrition and Bromatology, Pharmacy Faculty, Campus Vida, University of Santiago de Compostela, Santiago de Compostela 15782, Spain

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Different studies have already demonstrated a direct relationship between seaweed consumption and the prevention and/or improvement in the treatment of diseases related to oxidative stress. Algae have been used, therefore, in the investigation of bioactive compounds, with the aim of developing new medicines and healthy foods. This article presents the results of the analysis of vitamin C, vitamin E, fucoxanthin, chlorophyll *a*, pheophytin *a*, lutein, β-carotene, total polyphenol content, and antioxidant activity in *Himanthalia elongata* dehydrated, hydrated by immersion, boiled, and steamed. These results contribute to a better understanding of the bioactive compounds' behavior when subjected to different culinary methods and reaffirms the potential of *H. elongata* as interesting food in our diet.

Keywords: seaweed; *Himanthalia*; bioactive compounds; culinary processes

Diferentes estudios han demostrado una relación directa entre el consumo de algas y la prevención y/o mejora en el tratamiento de enfermedades relacionadas con el estrés oxidativo. En las algas se ha investigado la presencia de compuestos bioactivos, con el objetivo de desarrollar nuevos medicamentos y alimentos para la salud. Este trabajo presenta los resultados del análisis de vitamina C, vitamina E, fucoxantina, clorofila *a*, feofitina, luteína, β-caroteno, contenido total de polifenoles y actividad antioxidante en *Himanthalia elongata* deshidratada, hidratada por inmersión, hervida y al vapor. Estos resultados contribuyen a una comprensión mejor del comportamiento de los compuestos bioactivos cuando la *H. elongata* se somete a diferentes métodos culinarios y confirma su interés potencial como alimento de nuestra dieta.

Palabras claves: algas; *Himanthalia*; compuestos bioactivos; procesos culinarios

Introduction

Algae are organisms that live in complex habitats submitted to extreme conditions (for example: changes of salinity, temperature, nutrients, or UV–VIS irradiation), and therefore they must adapt rapidly to new environmental conditions to survive, producing a great variety of secondary (biologically active) metabolites which cannot be found in other organisms. Also, considering their great taxonomic diversity, investigations related to the search for new biologically active compounds from algae can be seen as an almost unlimited field. For this reason, algae can be a very interesting natural source of new compounds with biological activity that could be used as functional ingredients (Plaza, Cifuentes, & IBANEZ, 2008). Traditionally, seaweeds have been exploited mainly for the industrial production of phycocolloids, such as agar-agar, alginate, and carrageenan, used as gelling and thickening agents in the food or pharmaceutical industries and not as culinary items or for recovering beneficial biomolecules. Recently, macroalgae have been used as a novel food with potential nutritional benefits in food industry as a valuable ingredient in any dish (soups, rice, pasta, pâté, appetisers, biscuits, etc.) and medicine, since current research on mammals, trials on humans, and epidemiological studies have revealed their potential as a rich source of natural bioactive compounds with antiviral, antifungal, antibacterial, antioxidant, anti-inflammatory, hypolipidemic, and antineoplasteic properties (Mohamed, Hashim, & Rahman, 2012; Shalaby, 2011). Thus, there is a growing interest in research into bioactive compounds in different algae species and a lack of reports, especially on changes in the content of their bioactive compounds after culinary treatments. In fact, research into bioactive compounds focuses mainly on macroalgae species typical of Asian and Australian coasts (Ferraces-Casais, Lage-Yusty, Rodríguez-Bernaldo de Quirós, & López-Hernández, 2012) and up until now, very few articles have reported on the changes of bioactive compounds in seaweeds. In view of the fact that seaweeds are an important source of bioactive compounds in a human diet and that culinary treatment could affect their content, the aim of this work was to study the influence of three different culinary treatments over the bioactive compound content of *Himanthalia elongata* from Galicia, Northwest Spain.

Material and methods

Samples

Six packages of dehydrated brown seaweed *H. elongata* of different suppliers were purchased from a local supermarket in the city of Santiago de Compostela, Spain. This macroalga was collected on the Atlantic coast region of Galicia (NW Spain).

*Corresponding author. Email: julia.lopez.hernandez@usc.es

The cooking process for boiled *H. elongata* (15 min in boiling water at 100ºC) and rehydrated *H. elongata* (submerged 10 min in water) was done following the instructions on the product label. For the steamed algae, 40 min was established in our laboratory as the time needed for the algae to reach softness.

Ascorbic acid

The methods used are similar with very slight modifications to those reported by Amorim, Lage-Yusty, and López-Hernández (2012) and Lage-Yusty, Alvarado, Ferraces-Casais, and López-Hernández (2013). Briefly, for determination of ascorbic acid, seaweed was extracted with metaphosphoric acid and analyzed by high-performance liquid chromatography (HPLC) with analytical column C18, mobile phase consisting of acetic acid in water 0.1% (v/v). Detection wavelength was 245 nm, and the calibration was performed by external standard method (0.5–5 mg/L), with a coefficient of determination 0.9972.

Vitamin E and color compounds

In the analysis of vitamin E and color compounds by HPLC, 2 g of seaweed sample was extracted with methanol–hexane–dichloromethane (50:25:25, v/v/v) and acetone. Color compounds were determined at 450 nm using variable wavelength detector, and vitamin E was estimated using fluorescence detector set at λ_{ex} = 288 nm; λ_{em} = 331 nm. Chromatographic analysis was performed using a mobile phase of three solvents: methanol, acetonitrile, and hexane–dichloromethane (50:50, v/v) in gradient elution. Quantification was carried out with the external standard method, and the coefficient of determination was in the range of 0.9971–0.9999.

Polyphenols and antioxidant activity

To determine polyphenols and antioxidant activity, seaweed samples (1–1.2 g) were extracted with 10 mL of methanol–water–acetic acid (30:69:1, v/v/v) and acetone 70% (v/v); the supernatants were then combined and made up to 20 mL with Milli–Q water.

The antioxidant activity has been evaluated by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method (Amorim et al., 2012). Trolox was used as a standard to construct the calibration curve. Results were expressed as µM Trolox equivalents.

The total polyphenol content was determined using the Folin–Ciocalteu method (Amorim et al., 2012) at 720 nm after 30 min in the dark. This method prevents potential interference by treating the sample with insoluble polyvinylpolypyrrolidone (PVPP), which specifically binds to the polyphenols in an acid medium. Thus, the absorbance difference between the treated and untreated extract with PVPP reflects the concentration of polyphenols (Parys et al., 2007). Phloroglucinol was used as a standard (10–100 mg/L, r^2 = 0.9982) to construct the calibration curve. Polyphenols and antioxidant activity determinations were performed by duplicate.

Statistical analysis

StatGraphics plus (Statpoint Technologies, Inc., Warrenton, VA, USA) software was used to perform one-way analysis of variance, and a least significant difference (LSD) test at a 95% to identify differences among samples. A multiple range test ($p < 0.05$) was also used to identify differences.

Results and discussion

The obtained results are shown in Table 1. The average value ± standard deviation (SD) was obtained for six samples and is expressed in mg/kg of sample in dry matter, except for antioxidant activity, which is expressed in µM Trolox/kg d.m. Comparing different culinary treatments with dry samples, boiled treatment has increased the value of the antioxidant activity, fucoxanthin, β-carotene and pheophytin *a*, while steaming has increased pheophytin *a* content. Hydration by immersion, compared with other cooking processes, presented the highest results in every analyzed compound, except for pheophytin *a* and β-carotene, which were higher in the boiled samples. Hydration by immersion was the unique treatment that presented values of vitamin E and chlorophyll *a*. Vitamin C was not detected after cooking process. The multiple correlation coefficients between the antioxidant activity values in all culinary methods and all the analyzed compounds showed a good correlation r^2 of 0.95.

Antioxidant activity values were higher after boiling than the values of the dried samples. This result supports the study conducted by Rajauria, Jaiswal, Abu-Ghannam, and Gupta (2010) in which values for DPPH scavenging in *H. elongata* increased after thermal processing at similar temperature and time exposure conditions (95ºC for 15 min). Similar results were found by Cox, Gupta, and Abu-Ghannam (2012), who has studied the effect of the temperature on the phytochemical constituents of the dried edible Irish seaweed *H. elongata* and noted that for all of the rehydration temperatures analyzed, the radical scavenging activity increased up to 20 min of treatment in the range of 13.2–24.3%. However, the antioxidant activity in steamed samples was not statistically different from the dried samples, which suggests that the time of exposure had influenced negatively the contents of the antioxidant compounds, since steamed samples were submitted to a longer time of cooking (40 min). Cox,

Table 1. Antioxidant activity and bioactive compounds content (±SD) in six samples of *H. elongata* ± SD. Values are expressed as mg/kg dry matter, excepting antioxidant activity which is expressed in µmol Trolox/kg dry matter.

Tabla 1. Actividad antioxidante y contenido de compuestos bioactivos (±SD) en 6 muestras de H. elongata ± desviación estándar. Los valores se expresan en mg/kg de materia seca, excepto la actividad antioxidante que se expresa en µmol Trolox/kg de materia seca.

	Antioxidant activity	Phloroglucinol	vitamin C	Fucoxanthin	Lutein	vitamin E	β-carotene	Chlorophyll *a*	Pheophytin *a*
Dried	7392.4 ± 287.7^{a}	2041.6 ± 463.0$^{a.b}$	20.7 ± 9.2	51.3 ± 9.7^{a}	2.0 ± 1.2^{a}	–	9.5 ± 3.2^{a}	42.9 ± 25.8	474.9 ± 114.5
Rehydrated	20,981.8 ± 4285	3963.0 ± 1416	–	106.0 ± 21.5^{b}	4.6 ± 2.3^{b}	0.72 ± 0.42	17.3 ± 5.1$^{b.c}$	236.0 ± 66.0	1536.0 ± 166.7
Boiled	16,137.1 ± 3604	2528.1 ± 460.5^{a}	–	95.4 ± 8.7^{b}	–	–	22.0 ± 4.6^{b}	–	2314.0 ± 252.2
Steamed	7973.8 ± 1157^{a}	1234.9 ± 243.2^{b}	–	51.2 ± 18.3^{a}	3.0 ± 2.2$^{a.b}$	–	14.0 ± 4.1$^{a.c}$	–	1271.0 ± 206.5

Note: Same letters within same column are indications of not significant differences ($p < 0.05$).

Abu-Ghannam, and Gupta (2012) have reported that a combination of drying of *H. elongata* followed by boiling, the most significant increase in antioxidant activity. In contrast to the results obtained by Rajauria et al. (2010), which observed an increase in polyphenols content by thermal processing at 95ºC during 15 min, in this present study, no heat treatment has increased the polyphenols content, which suggests a possible difference between the methodological techniques applied or differences between seaweed environmental conditions in both studies. Nonetheless, these reductions are similar to those reported by Mazzeo et al. (2011), who observed a decrease in the total polyphenol content caused by cooking in water of vegetables such as spinach, cauliflower, and carrots. Thermorehydration also resulted in a steep decrease in the total polyphenolic content of the dried seaweed *H. elongata* within the first 10 min at each of the test temperatures (20, 40, 60, 80, and 100ºC) (Cox et al., 2012). Similarly, Turkmen, Sari, and Velioglu (2005) have also reported a decrease in the total polyphenol content after cooking of various vegetables such as squash, peas, leeks, and broccoli.

Comparing all treatments applied, a rehydrating treatment showed its protective effect on the bioactive compounds of *H. elongata*, presenting the highest values of antioxidant activity and total polyphenols content, which were negatively affected by the heating.

Compared with dried samples, values for pheophytin *a* were higher in all treatments used. Among these treatments, the highest values were observed in boiled and steamed samples, indicating that both heat treatments increased the pheophytin *a* values. Lower values of chlorophylls and increased values of pheophytin *a* in cooking processes are due to the production of pheophytin by the substitution of Mg^{++} by H^{+} in the chromophore group (porphyrin ring) of the chlorophyll under acidic conditions (Gandul-Rojas, Roca, & Gallardo-Guerrero, 2012) during the cutting and cooking of plant foods, when there is a release of intracellular enzymes and acids that can act intimately with chlorophyll–protein complexes (Chen & Huang, 1998).

Decreasing values for β-carotene were observed after boiled, rehydrated samples and steamed samples, respectively. A possible explanation of the higher content of β-carotene after boiling treatment could be the formation of Z (*cis*) isomers generated during the cooking from the degradation of the all-*trans* isomers. Recently, Imsic, Winkler, Tomkins, and Jones (2010) have observed that the cooking of carrots has produced (15 Z)-β-carotene isomers, a fact which was not observed in raw carrots. This isomerization occurs when the temperature employed is high enough to induce the dissolution of the crystal structure of the β-carotene (Imsic et al., 2010). It is well reported that after boiling, β-carotene content shows an increase (Azizah, Wee, Azizah, & Azizah, 2009; Bernhardt & Schlich, 2006; Hwang & Kim, 2013; Kidmose, Christensen, Agili, & Thilsted, 2007). However, different results showing decreased values after boiling can be found elsewhere (Deol & Bains, 2010; Rahman, Wahed, & Ali, 1990; Wu et al., 2008; Zhang & Hamauzu, 2004). In contrast to rehydrated and boiled samples, values of β-carotene in the steamed samples were not statistically different from the dried samples. Studies pertaining to the effect of steam on β-carotene content are controversial. Bernhardt and Schlich (2006) reported lower values in β-carotene after steam treatment, and Ruiz-Rodriguez, Marín, Ocaña, and Soler-Rivas (2008) observed 15% of β-carotene loss in pumpkin steamed at the same cooking conditions of this present study (100ºC for 40 min), De La Cruz-García et al. (1997) found higher β-carotene values after steaming treatment in green beans.

For lutein, significantly higher values were observed only in rehydrated samples. Other treatments applied were not statistically different from the dried samples. Reports on the stability of the lutein were carried out by Delchier, Reich, and Renard (2012), who noted that boiling and steaming did not reduce lutein concentration in green beans and spinach. Kao, Chiu, Tsou, and Chiang (2012) have also observed that boiling caused almost no significant effect on (all-E)-forms of lutein. Mazzeo et al. (2011) found that lutein was not affected by boil or steam cooking processes in carrots, whereas boiling resulted in a significant decrease of lutein in spinach.

The variety of results concerning the thermal lability of carotenoids seems to be influenced by both experimental conditions and the nature of the food matrix, as many others authors have highlighted (Burgos et al., 2012; Kao et al., 2012; Perla, Holm, & Jayanty, 2012).

The fucoxanthin content was higher in rehydrated and boiled samples, while the values for steamed samples were not statistically different from the dried samples. There are few studies published on the behavior of the pigment fucoxanthin in food matrices subjected to thermal processing. In a ground-breaking report on the subject, Prabhasankar et al. (2009) observed that fucoxanthin is not degraded during the manufacturing of paste prepared with the wakame seaweed, even after cooking (with a decrease of less than 10%), indicating stability when mixed with gluten. Shang, Kim, Lee, and Um (2011) have used a technique that involves elevated temperatures for extracting fucoxanthin from seaweed *Eisinia bicyclis*, which revealed that heat increases fucoxanthin recovery. Kanazawa et al. (2008) have reported that the cooking of the fresh alga *Laminaria japonica* before the extraction of the fucoxanthin increased the content of this pigment by enzyme inactivation and that this procedure, therefore, suppresses the enzymatic decomposition of fucoxanthin.

Despite the increase in some bioactive compounds compared with dried samples, all the results presented in this study were inferior when compared with the contents of this fresh seaweed, except for pheophythin *a* (Ferraces-Casais et al., 2012) and dried immediately processed *H. elongate*, except for lutein (Lage-Yusty et al., 2013).

Conclusion

Results from the study showed that the rehydration of the seaweed *H. elongata* (without heating) is the best treatment applied prior its consumption to preserve its bioactive compounds and antioxidant activity. Among the heat treatments used, boiling has increased values for β-carotene, fucoxanthin and antioxidant capacity. On the other hand, the steam treatment has presented the most reduced values, save pheophytin *a*.

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