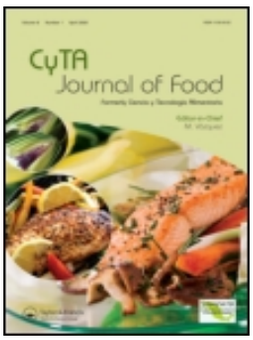

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SHORT COMMUNICATION

Changes in bioactive compounds content and antioxidant activity of seaweed after cooking processing

Cambios en compuestos bioactivos y actividad antioxidante de algas después del proceso de cocinado

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Wakame and Kombu seaweed, both fresh and reconstituted for consumption, were analyzed by HPLC for the determination of the content of ascorbic acid, vitamin E, β-carotene, lutein, fucoxanthin, chlorophyll *a* and pheophytin *a*; total polyphenol was done by Folin–Ciocalteau method and antioxidant activity by the DPPH test. Different behaviors have been observed on the contents of the bioactive compounds analyzed. Significant changes on ascorbic acid, β-carotene, lutein, fucoxanthin, chlorophyll *a*, pheophytin *a*, vitamin E, total polyphenols and antioxidant activity have been observed after the reconstitution of Wakame, whereas Kombu showed no significative variation of fucoxanthin, Vitamin E, total polyphenols and antioxidant activity. A high correlation between antioxidant activity and the above compounds was observed.

Keywords: antioxidants; total phenolic content; carotenoids; ascorbic acid; vitamin E; seaweeds

En este trabajo se ha determinado ácido ascórbico, vitamina E, β-caroteno, luteína, fucoxantina, clorofila *a* y feofitina *a* mediante HPLC, polifenoles totales por el método Folin–Ciocalteau y actividad antioxidante mediante la prueba DPPH, en algas Wakame y Kombu, frescas y reconstituidas para el consumo. Se han observado cambios significativos en el contenido de ácido ascórbico, vitamina E, β-caroteno, luteína, fucoxantina, clorofila *a* y feofitina *a*, polifenoles totales y actividad antioxidante después de la reconstitución del Wakame, mientras que el Kombu no presenta variaciones significativas para la fucoxantina, vitamina E, polifenoles totales y actividad antioxidante. Se ha encontrado una alta correlación entre los compuestos bioactivos analizados y la actividad antioxidante.

Palabras claves: antioxidantes; polifenoles totales; carotenoides; ácido ascórbico; vitamina E; algas

Introduction

In the last years, many marine resources have attracted attention in the search for bioactive compounds to develop new medicines and healthy food (Gopalraj, Karunamoorthy, Ganapathy, Fatimson, & Perumal, 2011). At present, due to the demand of natural products, seaweeds have attracted a big interest as a source of bioactive compounds with an important role in the development of nutraceutics. Algae are highly diverse in their composition, taxonomic family, habitat characteristics and period of harvesting (Pérez-Pérez, Rodríguez-Malaver, Padilla, Lapenna, & Medina-Ramírez, 2009) in addition to the changes caused by the process of being cooked before consumption.

The compounds with antioxidant activity in seaweed include carotenoids, vitamin E and chlorophylls and its derivatives, polyphenols or ascorbic acid (Plaza, Cifuentes, & Ibáñez, 2008; Yuan, 2007).

The aim of this work was to evaluate the bioactive compounds content and antioxidant activity in fresh and dried seaweeds that are subjected to boiling treatment, following the instructions of the manufacturer, and to establish the possible relations between the analyzed compounds and the antioxidant activity.

Materials and methods

Chemicals and standard solutions

All chemicals used were of analytical grade. Acetone, ethanol, methanol, hexane, HCl, dichloromethane and metaphosphoric acid were from Merck (Darmstadt, Germany). Sodium carbonate decahydrate ($Na_2CO_3 \cdot 10H_2O$) and acetic acid were supplied by Riedel-de Häen (Seelze, Germany). Fucoxanthin was obtained from Carotenature (Switzerland). Folin–Ciocalteu's reagent, the standards of phloroglucinol, DPPH (2,2-Diphenyl-1-picrylhydrazyl) and (+/−) α-Tocopherol were from Fluka, Biochemika and were obtained from Sigma-Aldrich (Steinheim, Germany), and ascorbic acid, Chlorophyll *a*, β-carotene and Trolox (6-hydroxy-2,5,7,8-tetra-methylchroman-2-carboxylic acid) were from Sigma-Aldrich (Steinheim, Germany).

The water used for all solutions was obtained from a Milli-Q water purification system (Millipore) (Bedford, MA, USA).

Stock standard solutions of vitamin C, carotenoids and vitamin E, phloroglucinol and Trolox were prepared in aqueous 4.5% metaphosphoric acid, acetone 70% (v/v) acetone and methanol, respectively.

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Samples

Fresh brown seaweeds used in this work were *Laminaria* sp. and *Undaria pinnatifida*. Samples were collected by the factory Algamar (Redondela, Pontevedra, Spain) in February 2011 and a lot was sent immediately for analysis. Another lot, in the factory was dried at 45°C for 24 h and then stored at room temperature for 12–15 hr. Then they are packed in polypropylene bags, and sent to laboratory. The two macroalgae species were collected on the Atlantic coastal region on Galicia (NW Spain).

The preparation of algae was carried out following the instructions on the nutrition label on the packaging of algae consisting of cooking in boiling water for 20 min for algae *Undaria pinnatifida* (Wakame) and 1 hr for *Laminaria* sp. (Kombu).

Determination of ascorbic acid

For the extraction of ascorbic acid from the samples, 1.0–1.5 g was weighed into a conical flask protected from light by covering with aluminum foil. About 10 ml of aqueous 4.5% metaphosphoric acid was added to the seaweed. The mixture was vortexed (Ika Vortex VG3 Ika-Werke Staufen, Germany) for 15 min and then centrifuged (Eba 12 centrifuge, Hettich, Kirchlengern) at 5500 rpm for 2 min. The residue was re-extracted with the same procedure.

The supernatants of the extractions were filtered and then diluted to 25 ml with Milli-Q water. An aliquot of the final solution was filtered through a 0.45 μm Millipore disposable filter, keeping the sample away from direct sunlight and then injecting into Spectra-Physics liquid chromatograph, equipped with a Jasco PU1580 quaternary pump and a Rheodyne 20 μl injection loop. The optical scanning detector was a Spectra Focus UV-VIS, controlled by Jasco Chrompass Chromatography Data system for Windows (version 1.7.403.1). The analytical column was a Kinetex C_{18} (150 × 4.6 mm, 2.6 μm, Phenomenex®, Torrance, CA 90501-1430, USA). A mobile phase consisting of acetic acid:water (1:999) (v/v) at flow rate of 0.7 ml/min was used. Detection wavelength was 245 nm and the calibration was performed by external standard method. The water used for preparing all solutions was obtained from a Milli-Q water purification system (Millipore). Quantification was carried out with the external standard method (0.5–5 mg/l). The coefficient of determination is 0.9972.

Determination of carotenoids and vitamin E

Two grams of sample was extracted using 8 ml of methanol–hexane–dichloromethane (50:25:25, v/v/v) by agitation in a vortex (Autovortex SA6; Stuart Scientific, Redhill, UK) for 1 min protected from light and then centrifuged (Eba 12 centrifuge; Hettich, Kirchlengern) at 1500 rpm for 4 min. The extraction is repeated twice with 8 ml of acetone to 100%. Supernatants from the three extractions were diluted to 25 ml with acetone, filtered and injected into the chromatograph. The HPLC system consisted of an HP1100 quaternary pump, an HP1100 degassing device, autosampler Agilent 1200, a 20 μl injection an HP1100 VWD detector and an HP1100 fluorescence detector (San Jose, CA, USA).

The HPLC was controlled by Agilent ChemStation software. The separation was performed on a Teknokroma Tracer Extrasil ODS2 column (250 mm × 4.6 mm, 5 μm particle size). The column was thermostatized at 30°C.

Carotenoids were determined at 450 nm using variable wavelength detector and vitamin E was estimated using fluorescence detector set at $\lambda_{ex} = 288$ nm and $\lambda_{em} = 331$ nm. Chromatographic analysis was performed using a mobile phase consisting of methanol, acetonitrile and hexane–dichloromethane (50:50, v/v) in gradient elution. The flow rate is 1 ml/min. Identification of peaks was carried out by comparison of their retention times and diode-array spectra, taken at real time of analysis, with the corresponding data obtained by analyzing standard compounds. Quantification was carried out with the external standard method. All compounds showed a good linearity, coefficients of determination were in the range of 0.9971–0.9999.

Determination of antioxidant activity and total phenolics content

Each seaweed sample (1–1.2 g) was extracted in Pyrex-tubes with PTFE-lined screw caps, with 10 ml of methanol–water–acetic acid (30:69:1, v/v/v) and was shaken for 1 h in a vortex (Ika Vortex VG3 Ika-Werke Staufen, Germany). The tubes were then centrifuged (Eba 12 centrifuge; Hettich, Kirchlengern, Germany) at 302*g* for 4 min. The supernatant was removed and the residue was re-extracted with 10 ml of 70% (v/v) acetone; the supernatants were combined and made up to 20 ml.

DPPH method

Radical scavenging activities of seaweed extracts were measured using the method reported by Rodríguez-Bernaldo de Quirós, Lage-Yusty, and López-Hernández (2009) with a slight modification. Briefly, 150 μl of sample extract was added to 3 ml of a DPPH methanolic solution (60 mM). The absorbance at 515 nm was measured at $t = 0$ min and after 30 min, time taken to reach the plateau. Trolox was used as reference. Standard trolox solutions were also tested against the radical (10–200 μM) ($r^2 = 0.9961$). The $\%\Delta_{515}$ was calculated as follows $\%\Delta_{515} = [(A_{515t0} - A_{515t30})/(A_{515t0})] \times 100$. Results were expressed as μM Trolox equivalents. All determinations were performed by duplicate.

Total polyphenolics content

Total polyphenolics content of the seaweed extracts was determined according to the Folin–Ciocalteu's method. This reagent determines the reducing substances in the sample, and is not specific for phenolic compounds. Under slightly acidic conditions, PVPP is used specifically to absorb the polyphenols (Parys et al., 2007).

Adsorption of phenolic compounds with PVPP

Five millilitres of the extract was mixed with 100 mg of PVPP (30 mg/ml) and was shaken for 15 min (Ika Vortex VG3 Ika-Werke Staufen, Germany). It was allowed to rest for 3hr as a minimum, and then centrifuged (Eba 12 centrifuge, Hettich, Kirchlengern) at 4058 g for 5 min.

About 0.5 ml of extract (before and after removing polyphenols) was added to 4 ml of 6% sodium carbonate solution and 200 μl of Folin–Ciocalteu's reagent; the solution

was vortex-mixed (Autovortex SA6, Stuart Scientific, Redhill, UK) and incubated for 30 min at room temperature in darkness.

The absorbance of the sample was measured at 720 nm against a blank (0.25 ml of extraction solvent: methanol–water–acetic acid 30:69:1, v/v/v and 0.25 ml of acetone–water 70:30, v/v) + 4 ml of 6% sodium carbonate + 200 μl of Folin–Ciocalteu's reagent. The difference between the measurement before and after removing polyphenols is equivalent to the phenolic compounds content. Phloroglucinol (the basic structural unit of phlorotannins) was used as a standard (2.5–75 mg/l, $r^2 = 0.9998$) to construct the calibration curve.

Statistical analysis

PASW Statistics 18 statistical software was used to perform one-way analysis of variance (ANOVA) and a least significant difference (LSD) test at 95% was also used to identify differences among raw and cooked samples.

A Student's *t*-test ($p < 0.05$) was also used to identify differences between fresh and cooking samples.

Microsoft Office Excel 2007 was used to evaluate multiple correlations.

Results and discussion

The obtained results are shown in Table 1. The average value ± SD was obtained for $n = 6$ samples. The values are expressed in mg/kg of sample in dry matter. Fresh Wakame has presented the biggest value for the total antioxidant activity of the seaweed analyzed, followed by boiled Wakame. Significant differences were not observed for freshly boiled Kombu, whereas significant differences were observed ($p > 0.05$) for fresh and cooked Kombu. Jiménez-Escrig, Jiménez-Jiménez, Pulido, and Saura-Calixto (2001) reported that antioxidant activity and total polyphenols were reduced by the effect of dried seaweed.

The results indicate a significant loss of content in polyphenols for seaweed Wakame and a light increase for the seaweed Kombu after the reconstitution for consumption. Nevertheless, in Kombu they are not statistically significant.

These results are similar to that reported by Mazzeo et al. (2011) who observed a decrease of the content in polyphenol compounds in boiled carrot, spinach and cauliflower, among cooking treatment. Turkmen, Sari, and Velioglu (2005) also have reported that cooking caused loss of phenolics compounds in squash, peas and leek. Nevertheless cooking was found to give rise to an increase in phenolics in green beans, pepper and broccoli. These results are similar to that reported by Mazzeo et al. (2011) who observed a decrease of the content in polyphenol compounds in carrot, spinach and cauliflower, after cooking. Turkmen et al. (2005) also reported a loss of phenolic compounds in cooked squash, peas and leek; nevertheless, they found an increase in phenolics in green beans, pepper and broccoli after cooking.

Thus, it is assumed that the minor loss of polyphenols found in Kombu could be due to their polyphenol composition which is more stable to heat treatments.

The average vitamin C content of fresh Wakame is 118 mg/kg d.w. while cooking depletes the vitamin C content due to the instability of vitamin C at the drying and cooking temperature. Kombu has no detectable vitamin C content.

The majority of the studies that have related the losses of vitamin C with culinary treatments have shown that cooking in water produces losses of up to 75%. The decrease of the content in vitamin C of the vegetables also depends on the quantity of present oxygen, causing its oxidation (Lešková, Kubíková, Kováčiková, Košická, & Porubská, 2006).

There are significant differences between the values of vitamin E on Wakame and Kombu cooked and fresh. However, reconstituted Wakame has a lower content of vitamin E in relation to fresh Wakame, while in Kombu the content was increased by culinary treatment. According to Gliszczynska-Swiglo et al. (2006), cooking with water (boiling) in broccoli, causes a significant release of tocopherol. Bernhardt and Schlich (2006) assume that cooking in boiling water also leads to better availability of tocopherol in some foods, depending on the structure.

This could suggest that the more rigid structure of the seaweed Kombu is responsible for the observed differences in the vitamin E content between the two algae during cooking. This cell structure may have been softened by cooking and provided greater extractability, which would explain the higher content of vitamin E compared to fresh. In turn, it is assumed that the less rigid structure of Wakame is responsible for the exposure of vitamin E to thermal degradation and lower values of vitamin E in Wakame, while being cooked.

The results show a significantly higher level of β-carotene, fucoxanthin, and lutein in seaweed Wakame and Kombu cooked compared with fresh seaweed.

Table 1. Antioxidant activity and bioactive compounds content (±SD) ($n = 6$) in seaweed analyzed.

Tabla 1. Actividad antioxidante y contenido en compuestos bioactivos (±SD) ($n = 6$) en las algas analizadas. Los valores se expresan en mg/kg de muestra seca.

(mg/kg dry weight)	Wakame fresh	Wakame cooking	Kombu fresh	Kombu cooking
Trolox*	12263.18 ± 1657.67	5351.6 ± 2201.30	4372.76 ± 1744.80	2828.96 ± 620.63
Phloroglucinol	3986.78 ± 507.27	2600.67 ± 547.47	1175.98 ± 265.53	1409.22 ± 415.22
Ascorbic acid	118.75 ± 22.1	nd	nd	nd
Vitamin E	153.59 ± 61.49	79.88 ± 12.77	9.42 ± 10.4	24.10 ± 4.74
Fucoxanthin	1119.13 ± 121.97	1699.95 ± 103.57	724.16 ± 231.68	839.15 ± 116.80
Lutein	8.41 ± 0.49	112.58 ± 7.61	nd	nd
β-Carotene	316.05 ± 10.20	898.66 ± 34.05	96.32 ± 20.35	200.31 ± 27.36
Chlorophyll *a*	7261.0674 ± 155.98	nd	4182.32 ± 511.18	nd
Pheophytin *a*	332.45 ± 15.04	10964.16 ± 1049.92	184.49 ± 16.1	4441.26 ± 534.19

Note: *μM/kg d.w.; nd, not detected. Values are expressed as mg/kg dry weight.

The increase in the carotenoid content observed in all algae can also be related to the increase in the extraction efficiency due to the release of carotenoids by heating of the binding protein (Rickman, Bruhn & Barret, 2007). For β-carotene it is known that cooking process can increase the extractability and thereby probably improves the bioavailability of β-carotene from the vegetable matrix (Bernhardt & Schlich, 2006).

Similar results are documented in the literature, such as the increase of the content in β-carotene and lutein observed by de la Cruz-García et al. (1997) and Gliszczynska-Swiglo et al. (2006), after the culinary treatment of green beans and broccoli, respectively.

Chlorophyll *a* has high values in the fresh seaweed Kombu and Wakame and is not detectable in cooked seaweed Kombu. On the other hand, content of derivatives of chlorophyll, pheophytin *a*, after the cooking process has been greater. The highest content of pheophytin in Wakame obviously explained by the higher chlorophyll content in relation to the fresh seaweed.

In the study of multiple correlation of antioxidant activity the following parameters were analyzed: total polyphenols, vitamin C, vitamin E, fucoxanthin, lutein and β-carotene. Multiple correlation coefficient: 0.930 ($r^2 = 0.865$).

The correlation between antioxidant activity and total polyphenols ($r^2 = 0.672$), and vitamin C + E ($r^2 = 0.732$) was reasonable.

Conclusions

The results of this study indicate that seaweeds can be considered as a good source of bioactive compounds with antioxidant activity.

There was a statistically significant correlation between antioxidant activity and bioactive compounds analyzed. Bioactive compounds have shown statistically significant changes by drying and cooking before consumption.

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