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Validation of a spectrophotometric methodology for a rapid iodine analysis in algae and seaweed casts

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ABSTRACT

Iodine plays an important role in human metabolism and its deficiency is particularly harmful in pregnancy and childhood. It remains a major public health concern in many countries, especially in Portugal. The main purpose of this work was to develop a validated spectrophotometric analysis for a fast and reliable iodine quantification in algal samples. Absorbance was determined at 410 nm demonstrating a good linearity ($R^2 \approx 1.0$) in the range of 0–0.06 mg I/100 g. LOD and LOQ were 1.7×10^{-3} and 5.0×10^{-3} mg I/100 g, respectively. Accuracy was determined using recovery and varied between 101 and 118%. For precision analysis, an intra-day test performance (RSD = 8.7%) and a repeatability assay (RSD = 3.8%) were performed. Matrix effect assessment demonstrated that this had a negligible effect (3.2%) in the iodine quantification. The spectrophotometric method was externally validated, for iodine quantification in algal samples, by INSA certified laboratory. The correlation coefficient between external iodine quantification and our work was $R^2 \approx 0.9$, showing a good correlation. Applicability was assessed in 25 macroalgae species (5 green, 9 red and 11 brown), 12 seaweed casts, collected in Canary Islands and 1 microalga (*Isochrysis galbana*) provided by ITC (Instituto Tecnologico de Canarias).

1. Introduction

In many areas of the world, soil surface is becoming gradually impoverished in iodine content due to leaching processes [5]. This important mineral is essential for human biochemistry and physiology due to its incorporation in thyroid hormones T3 (3,5,3-triiodothyronine) and T4 (thyroxine or 3,5,3,5-tetraiodothyronine), which, in turn, are responsible for the regulation of several processes of cellular metabolism and energetic balance, including mitochondrial metabolism, thermoregulation, and the catabolism of carbohydrates, lipids and protein [31]. Iodine is particularly needed during the early stages of growth and maturation of most organs [5]. Iodine is now recognized as playing a protective role against fibrocystic breast disease and breast cancer [24]. A relationship has also been hypothesized between iodine deficiency and a number of other health issues such as attention deficit hyperactivity disorder, psychiatric disorders [20] and nonspecific disease categories such as chronic fatigue and depressed immunity [6]. Due to the high iodine content in seaweed, its supplementation use increases the thyroid stimulating hormone (TSH) in healthy postmenopausal women [34] and slightly decreases serum free thyroxine FT4 [17,18].

Iodine deficiency remains a major public health concern in many countries, including some European ones [1,2]. In 2011, it was estimated that 44% of the European population, which would be about 393 million people, had insufficient iodine intake, evidenced by the concentration of urinary excretion < 100 µg/L [2]. Mainland Portugal and Portuguese islands are no exception. According to Limbert et al. [14], in a study with 3631 pregnant women, 83.2% in the mainland and 94.6% in Madeira and Azores islands have a urinary iodine content below 150 µg/L. The proximity to the sea does not prevent iodine deficiency. The same study was carried out on 311 children from Madeira Island and 676 children from Azores of both sexes, aged between 6 and 12 years, with 67.8% and 78.4%, respectively, showing insufficient iodine excretion. The high rainfall, with its leaching effect removing iodine from soils, may explain part of the iodine deficiency found. This gives seaweed great potential as a health promoting ingredient in the functional food industry [7]. Also, seaweed extracts could be used to bio-fortify soils and for animal production, allowing an indirect path for iodine supplementation of man food intake.

New analytical methods were developed over the years, with state-of-the-art equipment's that efficiently analyze the iodine content and determine the iodine speciation in several different biological samples. These methodologies include neutron activation analysis, atomic absorption spectrometry, inductively coupled plasma with optical emission spectrometry (ICP-OES), x-ray fluorescence, electrochemical, potentiometric probes and inductively coupled plasma with a mass

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spectrometer (ICP-MS) [3,10]. But all of these equipment's are not available for most of the laboratories, due to its prohibitive costs or maintenance. When iodine speciation is not required, the spectrophotometric method for iodine analysis in algae samples, properly validated, still represent a significant advantage due to its low cost and its fairly easy methodology.

The main purpose of this research was to conjugate previous published methodologies based on Sandell and Kolthoff [30] method for iodine quantification and develop a validated spectrophotometric method, based on available low-cost equipment present in most laboratories, to enable a rapid and reliable quantitation of the iodine content in algae samples.

2. Materials and methods

2.1. Seaweeds from Madeira Archipelago

Samples of 25 seaweeds were collected in a 10-meter maximum depth dive in the Madeira archipelago. The following green seaweeds (Chlorophyta) were collected: *Caulerpa racemosa* (Forsskål) J. Agardh 1873: 35, *Dasycladus vermicularis* (Scopoli) Krasser in Beck & Zahlbruckner 1898: 459, *Ulva intestinalis* Linnaeus 1753: 1163, *Ulva lactuca* Linnaeus 1753: 1163 and *Ulva* sp. Red seaweeds (Rhodophyta) comprise *Asparagopsis armata* Harvey 1855: 544, *Asparagopsis taxiformis* (Delile) Trevisan 1845: 45, *Chondrus crispus* Stackhouse 1797: xxiv, *Corallina officinalis* Linnaeus 1758: 805, *Galaxaura rugosa* (J.Ellis & Solander), *Grateloupia lanceola* (J.Agardh) J. Agardh 1851: 182, *Halopithys incurva* (Hudson) Batters 1902: 78, *Laurencia obtusa* (Hudson) J.V.Lamouroux 1813: 130 and *Nemalion elminthoides* (Velley) Batters 1902: 59. Brown seaweeds (Phaeophyta) include *Cystoseira compressa* (Esper) Gerloff & Nizamuddin 1975: 342, *Cystoseira humilis* Schousboe ex Kützing 1860: 18, *Cystoseira usneoides* (Linnaeus) M. Roberts 1968: 259–261, *Dictyopteris polypodioides* (A.P.De Candolle) J.V.Lamouroux 1809: 332, *Dictyota dichotoma* (Hudson) J.V.Lamouroux 1809: 42, *Halopteris filicina* (Grateloup) Kützing 1843: 293, *Halopteris scoparia* (Linnaeus) Sauvageau 1904: 349, 377, *Lobophora variegata* (J.V.Lamouroux) Womersley ex E.C.Oliveira 1977: 217, *Padina pavonica* (Linnaeus) Thivy in W.R.Taylor 1960: 234, *Sargassum vulgare* C. Agardh 1820: 3 and *Zonaria tournefortii* (J.V.Lamouroux) Montagne 1846: 32.

The samples were collected in summer and transported in seawater to laboratory and gently rinsed with filtered fresh water. Afterwards, the seaweeds were frozen at −35 °C and freeze-dried under reduced pressure (4×10^{-4} mbar), with a cooling trap set at −56 °C for 5 days. Lyophilized samples were milled to 200 mesh particle size, vacuum packed and stored at −35 °C until use.

2.2. Seaweed beach cast and Isochrysis galbana

Seaweed beach casts were collected in Canary Islands, in the north shore of the island of Gran Canaria in “Playa de Las Canteras”, from November 2016 till October 2017. These were collected in the same day of appearance, transported, washed with fresh water and sun-dried. Also, two lots of *Isochrysis galbana* Parke 1949:265, a brown microalgae (Haptophyta) cultivated and harvested at the “Instituto Tecnológico de Canarias” (ITC) were included in this study. *I. galbana* was originally cultivated in a chamber with two posterior inoculations to small scale raceways and finally transferred into a 6000 L raceway inside a greenhouse with light intensity varying between 400 and 1700 μmols photons $m^2 s^{-1}$. Culture medium was a standard f/2 composed in seawater.

2.3. Iodine quantification

Iodine assessment was initiated with seaweed incineration, adopting the method described by Mahesh et al. [15] with modifications. About 0.5 g of sample was weighted into porcelain crucibles and 0.5 mL of potassium hydroxide solution (6 M) mixed with a metal rod and placed in an oven at a temperature of 95 °C, during 1 h. Afterwards, 0.5 mL of zinc sulphate solution (0.5 M) was added and the sample returned to the oven for a 1 h at 95 °C. The crucibles were then placed in a muffle furnace to reach the 600 °C for a period of 1 h. After, it was reconstituted in deionized water, filtered to remove particles and the volume adjusted accordingly. The oxidation process and spectrophotometric measurement were performed according to Pino et al. [26] with modifications. Aliquots of 0.2 mL of sample or standard were added to test tubes, followed by addition of 1.0 mL of ammonium persulfate (1 M). Samples underwent a process of oxidation during 30 min in a water bath at 95 °C. Subsequently, 2 mL of arsenic acid (25.3×10^{-3} M), 1 mL of sulfuric acid (1.3 M) and 1 mL of water were sequentially added. The tubes were placed in a water bath at 32 °C for 10 min, then 0.5 mL of ceric ammonium sulphate (15.8×10^{-3} M) was added, vortexed and placed again in the water bath for 10 min. A standard solution A performed with KIO_3 to a concentration of 7.9 M (1000 μg/mL). Standard solution B prepared by diluting the standard solution A to 100 times to an iodine concentration of 78.7 mM (10.0 μg/mL). Working standards with concentration of 0.8, 1.6, 2.4, 3.2, 3.9 mM were prepared in distilled water. After incubation the transmittance of the samples or standards was read at 410 nm in a spectrophotometer. The calibration curve was prepared daily by placing transmittance (%) against iodine concentration (mg of iodine per 100 g). All samples were analyzed in triplicates in all tests carried out.

2.4. Validation methodology

To validate the spectrophotometric iodine quantification method, linearity, sensitivity, accuracy, precision, matrix effect and applicability were determined. Linearity included a linear regression, linear concentration range and correlation coefficient. Sensitivity included limit of detection (LOD) and limit of quantification (LOQ). Accuracy was performed evaluating the recovery percentage of spiked samples, adding to the incinerated biomass standard solutions of KIO_3 with several concentrations (0.01–0.05 mg I/100 g) to assess instrument recovery capability. Precision included the analysis of intra-day variability and repeatability and results are provided in relative standard deviation (RSD, %). Intermediate precision was performed calculating the recovery yield using different spectrophotometers (Shimadzu PC-1601 and Shimadzu PC-2401) and calibration curves in different days. Matrix effect (% ME) was also calculated to determine if the seaweed matrix interfered in the iodine extraction and this was calculated relating the slope of a fortified sample (before incineration) with different concentrations of KIO_3 and the slope of a standard curve of KIO_3 (Eq. (1)). Sample incineration was measured for its variability robustness, using different times. Applicability of the validated methodology was assessed in 26 algae and 12 seaweed beach cast samples. All samples were analyzed in triplicates in all tests carried out.

$$\%ME = \left(\frac{\text{slope fortified sample}}{\text{slope standard curve}} \times 100 \right) - 100 \quad (1)$$

Nine samples were assessed in triplicate by an external laboratory (INSA – Instituto Nacional de Saúde Dr. Ricardo Jorge), certified for iodine analysis in biological samples to determine the analytical deviation of the iodine content between an ultraviolet – visible (UV–Vis) spectrophotometric method (this work) and a validated inductively coupled plasma mass spectrometric method (ICP-MS - INSA).

2.5. Statistical analysis

All values are expressed as mean of three replicates ± standard deviation except for the LOD and LOQ assessment performed to *I. galbana*, which were performed ten replicates. The statistical data analysis

Table 1
Summarized validation results obtained for the iodine quantification through the spectrophotometric methodology.

	Parameter	Result
Linearity	Linear regression (y = mx + b)	703.6x + 34
	Linear concentration range	0–0.06 mg I/100 g
	R^2	1.0
Sensitivity	LOD (standard analytical solution 0.01 mg I/100 g)	1.7×10^{-3} mg I/100 g
	LOQ (standard analytical solution 0.01 mg I/100 g)	5.0×10^{-3} mg I/100 g
	LOD (sample with lowest iodine content, *Isochrisis galbana*, 10 readings)	6.0×10^{-2} mg I/100 g
	LOQ (sample with lowest iodine content, *Isochrisis galbana*, 10 readings)	1.8×10^{-1} mg I/100 g
Accuracy	Recovery	%
	SW + 0.01 mg I/100 g	103
	SW + 0.02 mg I/100 g	118
	SW + 0.03 mg I/100 g	104
	SW + 0.04 mg I/100 g	101
	SW + 0.05 mg I/100 g	102
Precision	Intra-day (% RSD)	8.7
	Repeatability (% RSD)	3.8
Matrix effect	Variation (%)	3.2

All determinations were carried out in triplicate except the LOD and LOQ using Isochrisis galbana, which 10 readings were performed.

was performed, using SPSS 24.0 program for Windows. Data were analyzed using one-way analysis of variance (ANOVA) and determined its homoscedasticity. Tukey's b test ($p \leq 0.05$) was performed to determine statistical variance between algae and seaweed beach casts. Also, using Excel from Microsoft Office 2013, we have performed several linear regressions.

3. Results and discussion

3.1. Methodology validation

Prior to the iodine content measurement in the selected algae and seaweed beach cast samples, the spectrophotometric method was properly validated (Tables 1 and 2). Linearity, sensitivity, accuracy, precision and matrix effect were determined at 410 nm wavelength, plotting transmittance against the concentration of iodine. Linearity was achieved plotting five different concentrations of KIO_3 and including a blank. The equation obtained was $y = 703.6x + 34$ with a correlation coefficient (R^2) close to 1.0 (Fig. 1). Linear concentration ranged between 0 and 0.05 mg of I/100 g. Sensitivity included a limit of detection (LOD) of 1.7×10^{-3} mg I/100 g and a limit of quantification (LOQ) of 5.0×10^{-3} mg I/100 g, when evaluating a macroalgae sample (*U. lactuca*), fortified with the lowest concentration of the standard analytical solution (0.01 mg I/100 g) and subjected to the entire procedure. The macroalgae iodine content is subtracted to the total iodine determined and LOD is calculated by multiplying the standard deviation (σ) by 3.3 and LOQ multiplying the standard deviation (σ) by 10. Also, we have calculated the LOD and LOQ, using the sample with the lowest iodine content (*I. galbana*), analyzed it 10 times and applying the same equations as before, the LOD was 6.0×10^{-2} mg I/100 g and LOQ 1.8×10^{-1} mg I/100 g. All samples were above the LOQ calculated in this work. Accuracy was performed, spiking incinerated samples with standard solutions of KIO_3 with several concentrations (0.01–0.05 mg I/100 g) and recovery values varied between 101 and 118%. Precision was determined calculating the intra-day variability of spiked samples, determining a value of 8.7% and repeatability using the same sample measured for five times in the same day resulted in a relative standard deviation (RSD) of 3.8%. The matrix effect was calculated to determine the interaction that seaweed matrix has on iodine assessment and found to have a 3.2% variability. Also, for validation purposes, we have performed the fortification of three macroalgae (1 green, 1 red and 1 brown) with 50, 100 and 200% of its iodine content, with KIO_3 standard solutions, before incineration (Table 2). This was performed to assess recovery of the added iodine, measuring the method efficiency. For 50% fortification, values determined were between 51.5 and 61%. When 100% fortification is implemented, 94.8 to 102.5% could be assessed. Highest fortification (200%), iodine recovery oscillated between 184.8 and 193.6%. These recovery values were considered suitable for the proposed methodology since the fortification process is implemented before incinerating the samples. The intermediate precision was evaluated by assessing the recovery yield, using the described method with two spectrophotometers, with different calibration curves in different days. The recovery yield graphic (Fig. 2) plots the theoretical *versus* real iodine concentrations with the measurement of concentration for each point. The obtained concentration represents the amount of iodine used in increasing fortification of spiked samples, with the amount of the seaweed iodine calculated and subtracted to the total iodine concentration. The R^2 for the four linear regressions was around 1.0 which resulted in an excellent fitting. The incineration time was tested for its robustness, to determine if incineration time would influence iodine content in seaweed samples (Table 3). The times tested were 1 h, 1:30 h, 2 h and 2:30 h. The average iodine content was 10.6 ± 0.3 mg I/100 g of dry weight (dw) with an RSD of 3%. These results demonstrated that samples incineration was complete with this methodology and that increasing the incineration time would not influence the mineralization of the samples. This assessment is of extreme importance, since some iodinated compounds could be volatized by the high temperatures. For biological matrices, dry incineration should be performed in the presence of alkaline agents to transform iodinated compounds into non-volatile species [13].

Table 2
Macroalgae samples fortified with standard analytical solutions of KIO_3 from 50 to 200% of its iodine content, before incineration.

Macroalgae	KIO_3 fortification (%)	Result (%)	Difference (%)
Ulva sp. (Green)	50	53.6	3.6 (+)
	100	94.8	5.2 (−)
	200	185.8	14.2 (−)
Grateloupia lanceola (Red)	50	51.5	1.5 (+)
	100	102.5	2.5 (+)
	200	193.6	6.4 (−)
Cystoseira usneoides (Brown)	50	61	11 (+)
	100	99.0	1 (−)
	200	184.8	15.2 (−)

All determinations were carried out in triplicate. Signal + or – indicate if the difference in the result % is positive or negative, respectively.

To validate the spectrophotometric methodology, the iodine content of nine algal samples was determined using inductively coupled plasma mass spectrometry (ICP-MS). The ICP-MS methodology have higher sensitivity and lower detection limit, and can be used to analyze different types of samples. Normally, the iodine is analyzed in alkaline medium, adding ammonia solution, to form a non-volatile NH_4I [3,10]. The comparison between these two techniques are described in detail in Table 4 and graphically represented in Fig. 3. The comparison of the iodine yields obtained by these two methodologies showed that they were very close, although some discrepancies were detected more prominently in *Galaxaura rugosa* and *Grateloupia lanceola*, with around 40% deviation between techniques. Judprasong et al. [11] also compared the ICP-MS and spectrophotometric technics, to assess the iodine content in some selected Thai foods, using a previous alkali ashing. It is referred in this work that some bias effect is to be expected from the spectrophotometric technic when iron and/or sodium is present, which can produce positive iodine content variance. This false positive effect is also described by Todorov et al. [35], which also compared these two technics to assess the iodine content in general food products. The

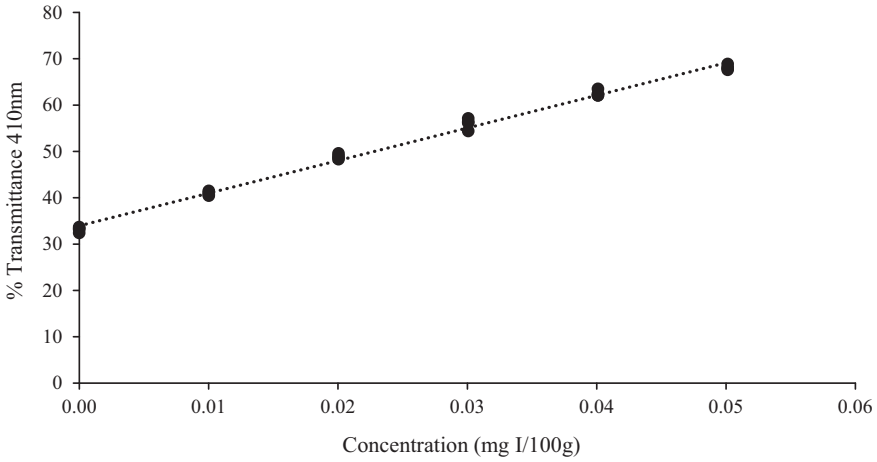

Fig. 1. Linear regression of potassium iodide determining the relation between concentration of compound (iodine) and transmittance (%) in the spectrophotometer. All determinations were carried out in triplicate.

measurement of remaining samples showed similar results of iodine content, with deviations between 8 and 17%, representing a positive validation of the spectrophotometric method. The linearity between spectrophotometric and ICP-MS results, using 8 samples, was strong with an R^2 around 0.9 (Fig. 3). The ninth sample that we have not introduced in the linear regression is the red macroalgae *A. taxiformis*. Due to its extreme high iodine content, it was not suitable for this regression. However, the iodine content determined by spectrophotometric and ICP-MS was 1162.7 ± 33.1 and 1268 ± 124 mg I/100 g dw, respectively. These results have a difference of 105.3 mg, around 9.1%, meaning that they are very similar to each other. Other methods for iodine quantification and speciation were developed and validated over the years. Nitschke and Stengel [22] developed an isocratic method using high performance liquid chromatography (HPLC) to quantify iodine in macroalgae and macroalgae products as iodide (I^-) with an ultraviolet (UV) detector set to 223 nm, with a previous dry alkaline incineration. Yeh et al. [36] also developed a validated methodology using gas chromatography linked to an electron capture detector (GC-ECD) to quantify iodine in macroalgae samples, previously derivatized with 3-pentanone. Sun et al. [33] developed a pressure-driven capillary electrophoresis methodology using an ion-pairing reagent to improve iodine speciation and discriminate between iodine containing molecules in macroalgae. This technique was externally validated with HPLC-ICP-MS, achieving good results.

3.2. Method applicability

Marine systems and organisms, such as seaweeds, contain and bio accumulate the majority of the available iodine [29]. Chemical iodine species in seaweeds seems to be mainly I^-, organic iodine and in minor amounts IO_3^- [8]. Twenty five seaweed samples, representative of the algal diversity of Madeira Archipelago, were assessed for their iodine content, comprising 5 green, 9 red and 11 brown seaweeds (Table 5). Green seaweed presented an average of 9.8 mg I/100 g dw, which varied between 2.6 ± 0.2 mg I/100 g dw and 22.0 ± 0.3 mg I/100 g dw, in *Ulva* sp. and *C. racemosa*, respectively. Hou and Yan [9], working with neutron activation analysis, have determined several inorganic elements in 35 seaweed species collected in China, in which *U. pertusa* contained between 1.3 and 3.3 mg I/100 g dw, *U. lactuca* 5.4 mg I/100 g dw and *U. intestinalis* 11.4 mg I/100 g dw. These results fit closely into the range described in this work. Red seaweed showed an average of 331.3 mg I/100 g dw, varying between 13.6 mg I/100 g dw and 1162.7 mg I/100 g dw, for *G. lanceola* and *A. taxiformis*, with the last specie presenting the highest iodine content. *A. taxiformis* was collected in three different locations of Madeira archipelago, two in Porto Santo and one in Madeira. Across sites, this alga specie, constantly presented considerably high iodine content when compared with the remaining

Table 3
Assessment of the iodine content, variating the time of incineration in the muffle oven at 600 °C, to test the applicability of the ultraviolet – visible (UV–Vis) spectrophotometry to seaweed matrices.

N°	Time of incineration	Mean ± SD (mgI/100 g of dw)	RSD (%)
1	1 h	10.3 ± 0.7	6.4
2	1 h30 m	10.4 ± 0.3	2.4
3	2 h	11.1 ± 1.2	6.6
4	2 h30 m	10.6 ± 0.5	4.7
Average		10.6 ± 0.3	3.0

Data are mean ± standard deviation in milligrams per 100 g of algae on a dry weight basis (dw). All determinations were carried out in triplicate.

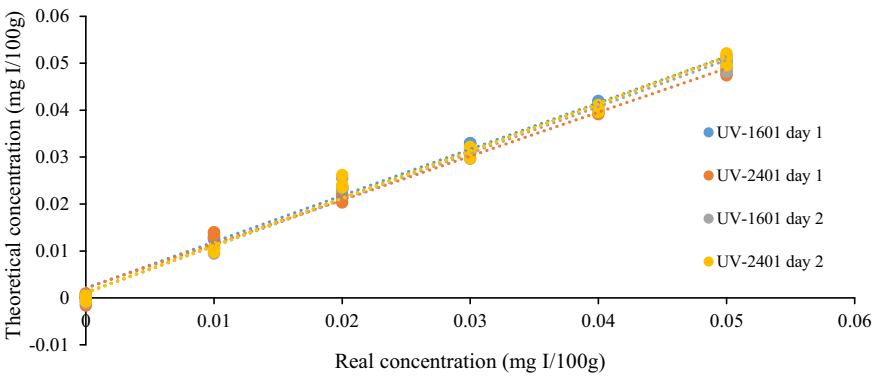

Fig. 2. Plot of theoretical and real (measured) concentration of iodine in the recovery yield testing. Spiked samples were measured using two different spectrophotometers (UV-1601 and UV-2401) in two different days. Values for plotting originate from subtracting the seaweed iodine content, remaining the fortified iodine content. All determinations were carried out in triplicate.

Table 4
Comparison of the iodine content of 9 selected macroalgae samples determined using UV–Vis spectrophotometry and ICP-MS. The ICP-MS analysis has performed with the assistance of INSA (Instituto Nacional de Saúde Dr. Ricardo Jorge).

Scientific name	Spectrophotometer (Our study)	ICP-MS (INSA)
	(mg I ± SD/100 g dw)	(mg I ± SD/100 g dw)
Asparagopsis taxiformis	1162.7 ± 33.1	1268 ± 124
Cystoseira humillis	6.8 ± 0.1	5.7 ± 0.2
Galaxaura rugosa	41.8 ± 0.9	24.5 ± 0.1
Grateloupia lanceola	13.6 ± 0.2	8.2 ± 0.5
Halopteris scoparia	71.3 ± 1.3	85.8 ± 8.1
Padina pavonica	6.7 ± 0.2	5.7 ± 0.2
Ulva intestinalis	6.3 ± 0.2	5.6 ± 0.3
Zonaria tournefortii	16.9 ± 0.3	15.4 ± 1.2
SC 14.11.2016	24.7 ± 0.4	27.7 ± 2.7

Data are mean ± standard deviation in milligrams per 100 g of algae on a dry weight basis. All determinations were carried out in triplicate. SC-Seaweed Cast. Instituto Nacional de Saúde Dr. Ricardo Jorge is a certified Portuguese institute for iodine analysis.

seaweeds, 936.4 ± 172.4 mg I/100 g dw. This specie was previously studied by Kaliaperumal [12] and identified as a "seaweed rich in iodine" used to ameliorate goitre disease. McConnell and Fenical [16] determined the biological purposes of the halogenated metabolites in *A. taxiformis*, which are kept in specialized gland cells [25], frequently functioning as protection compounds or as antioxidants. These are connected with environmental adaptations, since *A. taxiformis* usually grows where high number of seaweed herbivores occurs, in subtropical and tropical waters. Research performed in India reported a high iodine accumulation by *A. taxiformis*, 499.3 mg I/100 g dw, and *Asparagopsis* genus, 556.7 mg I/100 g dw [4]. Hou and Yan [9] also determined that *Corallina pilulifera* possess 16.1 mg I/100 g dw, very near to our values for *Corallina officinalis*, 18.1 ± 0.3 mg I/100 g dw, suggesting a close genetic relation in iodine bioaccumulation among seaweeds of this genus. Comparing with red seaweeds already in the food markets, these usually contain lower iodine concentration, between 7.2 and 29.3 mg I/100 g dw in *Palmaria* sp. [27] and 2.9 to 4.6 in *Porphyra* sp. seaweeds [36]. In this work, brown seaweeds presented an average iodine content of 30.1 mg I/100 g dw, ranging between 5.8 ± 0.2 mg I/100 g dw and 71.3 ± 1.3 mg I/100 g dw in *Cystoseira usneoides* and *Halopteris scoparia*, respectively. *Cystoseira* species presented the lowest iodine values in contrast to the *Halopteris* species. *Sargassum vulgare* included in this work had an iodine content of 62.3 ± 1.4 mg I/100 g dw very close to the lowest content described by Hou and Yan [9], which included in their work 6 *Sargassum* species varying from 11.1 to 593.9 mg I/100 g dw, demonstrating a very broad iodine content for this genera. These results are comparable with extensively commercialized brown seaweed species such as *Alaria* sp., ranging from 17.1 till 107 mg I/100 g dw, *Saccharina* genus, from 155.6 to 720.8 mg I/100 g dw [27], *Undaria* varying from 9.4 till 18.5 mg I/100 g dw and *Laminaria* ranging from 24.1 till 492.1 mg I/100 g dw [36]. For microalga *Isochrysis galbana*, the average value for iodine content was determined to be 0.32 ± 0.08 mg I/100 g dw, varying from 0.25 ± 0.03 mg I/100 g dw and 0.39 ± 0.04 mg I/100 g dw (Table 5). Although other works assessing *Isochrysis galbana* iodine content are not known, other microalgae species were already evaluated. For instance *Arthrospira platensis* Gomont, a cyanobacteria, was evaluated by Mosulishvili et al. [19] and was determined to reach a maximum of 200 mg I/100 g dw, but the evaluation performed by Romarís-Hortas et al. [28] only determined 1.2 mg I/100 g dw. Niedobová et al. [21] evaluated *Chlorella* genus using inductively coupled plasma - optical emission spectrometry (ICP-OES) in vacuum UV methodology and determined that iodine content can vary from 10 to 130 mg I/100 g dw. It seems that iodine content can fluctuate in microalgae depending in the abiotic and biotic factors inherent to their environment or cultivation conditions.

Twelve samples of seaweed beach casts, collected in Canary Islands, in the island of Gran Canaria, were evaluated for their iodine content. Iodine in these samples varied from 14.8 ± 0.7 mg I/100 g dw and 45.5 ± 1.5 mg I/100 g dw, averaging 32.2 ± 8.9 mg I/100 g dw (Table 6). Oyesiku and Egunyomi [23] evaluated pelagic masses of *Sargassum* collected offshore in Ondo State, Nigeria and determined that iodine content was 0.04 mg I/100 g dw. These seaweed beach casts, also known as "seaweed tides" or floating offshore masses of seaweed, directly harm traditional fishery [23], cause financial disorder to tourism and aquaculture productions, with increasing reports over the years, justifying the need to develop mitigation strategies to reduce the negative impact [32] and create new industrial applications for their economic development. Extracts of these seaweed masses or their direct application for soil fertilization, crop and animal production could be studied, introducing an iodine bio-fortification program to overcome iodine deficiency.

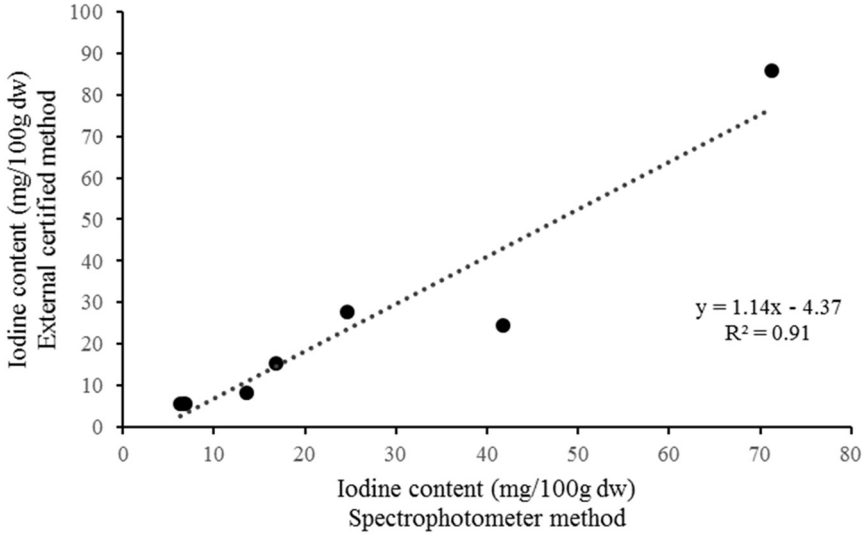

Fig. 3. Linear regression model determining the relation between the iodine content determined in 8 macroalgae samples analyzed by an external certified method, using an ICP/MS (INSA) and our work (spectrophotometer method). All determinations were carried out in triplicate.

Table 5
Iodine content in 26 species of algae, with 25 macroalgae collected in Madeira archipelago and 1 species of microalgae cultivated by ITC (Instituto Tecnologico de Canarias).

Scientific name	Color	Collection site	Island	Iodine (mg ± SD/100 g dw)
Caulerpa racemosa	Green	Calheta	Porto Santo	22.0 ± 0.3^{ab}
Dasycladus vermicularis	Green	Calhau da Serra de dentro	Porto Santo	5.3 ± 0.8^{a}
Ulva intestinalis	Green	Campanário	Madeira	6.3 ± 0.2^{a}
Ulva lactuca	Green	Santa Cruz	Madeira	12.9 ± 0.5^{ab}
Ulva sp.	Green	Porto das Salemas	Porto Santo	2.6 ± 0.2^{a}
Average				9.8
Asparagopsis armata	Red	Reis Magos	Madeira	938.7 ± 5.6^{e}
Asparagopsis taxiformis	Red	Abas do Rio	Porto Santo	833.1 ± 5.8^{d}
Asparagopsis taxiformis	Red	Praia do Zimbralinho	Porto Santo	813.5 ± 47.1^{d}
Asparagopsis taxiformis	Red	Reis Magos	Madeira	1162.7 ± 33.1^{f}
Corallina officinalis	Red	Praia do Zimbralinho	Porto Santo	18.1 ± 0.3^{ab}
Chondrus crispus	Red	Santa Cruz	Madeira	22.1 ± 0.7^{ab}
Galaxaura rugosa	Red	Reis Magos	Madeira	41.8 ± 0.9^{bc}
Grateloupia lanceola	Red	Campanário	Madeira	13.6 ± 0.2^{ab}
Halopithys incurva	Red	Abas do Rio	Porto Santo	42.5 ± 1.2^{bc}
Halopithys incurva	Red	Calhau da Serra de fora	Porto Santo	61.8 ± 0.7^{c}
Laurencia obtusa	Red	Porto das Salemas	Porto Santo	13.6 ± 0.5^{ab}
Nemalion elminthoides	Red	Baía D'Abra	Madeira	13.9 ± 0.6^{ab}
Average				331.3
Cystoseira compressa	Brown	Porto das Salemas	Porto Santo	13.2 ± 0.7^{ab}
Cystoseira humillis	Brown	Seixal	Madeira	6.8 ± 0.1^{a}
Cystoseira usneoides	Brown	Porto das Salemas	Porto Santo	5.8 ± 0.2^{a}
Dictyopteris polypodioides	Brown	São Vicente	Madeira	10.0 ± 0.1^{a}
Dictyota dichotoma	Brown	Abas do Rio	Porto Santo	8.2 ± 0.1^{a}
Halopteris filicina	Brown	Abas do Rio	Porto Santo	66.0 ± 0.4^{c}
Halopteris scoparia	Brown	Baía D'Abra	Madeira	71.3 ± 1.3^{c}
Lobophora variegata	Brown	Porto dos Frades	Porto Santo	64.2 ± 2.5^{c}
Padina pavonica	Brown	Seixal	Madeira	6.7 ± 0.2^{a}
Sargassum vulgare	Brown	Santa Cruz	Madeira	62.3 ± 1.4^{c}
Zonaria tournefortii	Brown	Reis Magos	Madeira	16.9 ± 0.3^{ab}
Average				30.1
Isochrysis galbana 12.05.2017	Brown	ITC	Gran Canaria	0.3 ± 0.0^{a}
Isochrysis galbana 26.05.2017	Brown	ITC	Gran Canaria	0.4 ± 0.0^{a}
Average				0.3

Data are mean ± standard deviation in milligrams per 100 g of algae on a dry weight basis. All determinations were carried out in triplicate. Different letters indicate significant differences ($p \leq 0.05$) determined in SPSS 24.0 using Tukey b test. SC-Seaweed Cast.

Table 6
Iodine content in Seaweed beach casts, collected in the island of Gran Canaria.

Sample	Iodine (mg/100 g ± SD dw)
SC 14.11.2016	24.7 ± 0.4^{a}
SC 29.05.2017	45.5 ± 1.5^{b}
SC 23.06.2017	31.0 ± 0.5^{c}
SC 26.06.2017	26.8 ± 0.8^{d}
SC 05.07.2017	35.8 ± 0.4^{e}
SC 12.07.2017	30.5 ± 0.6^{c}
SC 20.07.2017	39.0 ± 1.2^{f}
SC 08.08.2017	22.9 ± 0.4^{i}
SC 21.08.2017	14.8 ± 0.7^{j}
SC 18.09.2017	34.0 ± 0.6^{k}
SC 06.10.2017	40.5 ± 0.5^{fm}
SC 10.10.2017	41.2 ± 0.2^{m}
Average ± SD	32.2 ± 8.9

Data are mean ± standard deviation in milligrams per 100 g of seaweed beach cast on a dry weight basis. All determinations were carried out in triplicate. Different letters indicate significant differences ($p \leq 0.05$) determined in SPSS 24.0 using Tukey b test. SC-Seaweed Cast.

4. Conclusion

The iodine yield in algae is an important nutraceutical factor for humans, since this mineral is becoming scarce in terrestrial food due to lixiviation processes and intensive cropping. Consequently, algae could become a natural supplementation of this essential mineral, preventing iodine deficiency related disorders. The validated method performed in this study demonstrated a good working range (0–0.06 mg I/100 g), with an excellent correlation coefficient ($R^2 \approx 1.0$). Sensitivity was determined, achieving a low LOD (1.7×10^{-3} mg I/100 g) and LOQ (5.0×10^{-3} mg I/100 g) values, using the lowest concentration of the analytical standard solution. Also Accuracy was assessed calculating recovery and acceptable values were determined (101 and 118%). Precision was considered optimal, due to intra-day (RSD = 8.7%) and repeatability (RSD = 3.8%) assessment. Matrix effect was considered neglectable (3.2%) and considered not to imprint significant variation for iodine quantification. Also, fortification recovery assessment (50–200%), was performed to the 3 different macroalgae colours, adding KIO_3 standard solutions before sample mineralization. Results were considered suitable, since recovery % was not distant from the initial fortified value. This indicate that iodine volatilization in the incineration procedure, oxidation process and iodine dilution do not impose a significant variation. Iodine content in algae varies greatly between different species. This is demonstrated by the range of values obtained for macroalgae, between *Ulva* sp., 2.6 ± 0.2 and *A. taxiformis*, 1162.7 ± 33.1 mg I/100 g dw, with the other seaweeds being distributed between these values. This is a positive remark since it allows targeting seaweeds with a specific iodine content for precise market applications. These results suggest that *A. taxiformis*, a red macroalgae, is an iodine rich source, reaching values similar to a brown macroalgae known as Kelp, from the Laminariales. *Isochrysis galbana*, on the contrary, demonstrated a very low iodine content when compared with macroalgae and could be used for people with iodine sensibility. Seaweed beach casts varied their iodine content from 14.8 ± 0.7 to

45.5 ± 1.5 mg I/100 g dw, which is probably due to the seaweed species variations throughout the year. The external validation, performed with the INSA assistance was extremely helpful to determine the accuracy between very different methodologies for iodine quantification. These results showed that spectrophotometric method is still a reliable technique for rapid determination of iodine content in algae samples. With this work, it evidences the potential of using seaweed or beach cast, to produce bioactive extracts that could function directly or indirectly for iodine supplementation. These could be consumed directly by humans or applied in irrigated crops or even in animal production. It is therefore important to determine the iodine level present in each sample, using a robust and reproducible method, and in the present work we have proved that the method used herein has the qualities required for this purpose. Due to the prevalent awareness of this important micronutrient and nowadays potential of functional foods, these assessments are of great importance to prevent life threatening diseases, using local products or unused biomass.

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Declaration of author's contribution

1) Nuno Nunes contribution included conception and design of the study, performed the statistics and wrote the article.
2) Sofia Valente performed the analysis and interpretation of data.
3) Sónia Ferraz provided the study materials, obtained funding and revised it critically.
4) Maria do Carmo Barreto performed also the interpretation of data and revised the article critically.
5) M.A.A. Pinheiro de Carvalho helped with the conception and design of the study, obtained funding, revised it critically and provided the final approval.

Statement of informed consent, human/animal rights

No conflicts, informed consent, human or animal rights applicable.

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