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Evaluation of an *in vitro* method to estimate trace elements bioavailability in edible seaweeds

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ABSTRACT

Raw edible seaweed harvested in the Galician coast (Northwestern Spain), including two red seaweed types (Dulse and Nori), three brown seaweed (Kombu, Wakame and Sea Spaghetti), one green seaweed (Sea Lettuce) and one microalgae (*Spirulina platensis*) were studied to assess trace elements bioavailability using an *in vitro* method (simulated gastric and intestinal digestion/dialysis). Similarly, a cooked seaweed sample (canned in brine) consisting of a mixture of two brown seaweed (Sea Spaghetti and Furbelows) and a derived product (Agar–Agar) from the red seaweed *Gelidiumm sesquipedale*, were also included in the study. The total trace element content as well as the non-dialyzable fractions was carried out after a microwave acid digestion of the seaweed samples by inductively coupled plasma-mass spectrometry (ICP-MS). The dialyzable fraction was determined without any pre-treatment by ICP-MS.

PIPES buffer solution at a pH of 7.0 and dialysis membranes of 10 kDa molecular weight cut off (MWCO) were used for intestinal digestion. Accuracy of the method was assessed by analyzing a NIES-09 certified reference material (*Sargasso* seaweed). The accuracy of the in vitro procedure was established by a mass balance study which led to good accuracy of the whole *in vitro* process, after statistical evaluation (95% confidence interval). The highest dialyzability percentages ($100 \pm 0.2\%$) were obtained for Dulse in Mn and V.

1. Introduction

Chromium, cobalt, manganese and vanadium are essential trace elements for human health. Chromium is involved in cholesterol, lipid [1] and glucose metabolism [2] and has also been associated with the glucose tolerance factor (GTF) [3], the compound that increases the cellular absorption of insulin. This micronutrient has been associated with cardiovascular diseases [4].

The only nutritional function of cobalt is as an essential component of cobalamin or vitamin B12 and its deficiency is associated with anemia and neurological problems [5]. Manganese activates a great number of enzymes and is a constituent of some of them [6]. A balanced diet usually provides the manganese needed for normal function of the human organism, but its deficiency has been associated with malfunction of neuronal activity [7], impaired growth, osteoporosis and reproductive function [4].

Vanadium is an enzymatic cofactor that stimulates or inhibits enzymes and is associated with mimetic properties of insulin in diabetes mellitus [8] the vanadium toxicity is very low and there are no scientific evidences that show a correlation between damages in human health and chronic or accidental expositions to vanadium [9]. Vanadium [9], manganese and chromium [10] content in processed food tend to be higher than in natural products.

The ability of seaweeds to sorbs the metallic pollutants from the surrounding environment makes them highly suitable for using in biotreatment and bioremediation of contamined environment by heavy metals [11]. However if we think of seaweeds as a food stuff, the study of the presence of heavy metals is necessary to assess the safety of this food [12].

Dietary food sources of these essential elements are yeast, nuts, whole cereals, beer and wine for chromium [13]; meat, eggs and milk products for cobalt [14]; cereals, vegetables, wine, tea and coffee for manganese [10,15]; and shellfish, mushroom, parsley and pepper for vanadium [9].

Due to the ability of seaweed to concentrate inorganic species from seawater, they have been well recognized as a natural source of essential elements [16].

Although these marine products have been consumed in Asian countries since ancient times, the current interest in health foods in western countries has led to an increased presence of seaweed in western markets and also to the development of seaweed-based industries in Europe. This latter fact has led these industries to seek

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data on the concentrations of both essential and toxic elements before commercialization of the products. But in order to know the possible effects of trace elements present in foods, knowledge of the total content in the food is not sufficient; it is also necessary to know the bioavailable amount to humans.

Bioavailability is an important factor in nutrition because it varies with different foods and food components and with gastrointestinal conditions. Bioavailability depends on several processes such as digestion, absorption, transport, utilization and elimination.

To estimate the bioavailability of trace elements in human nutrition *in vivo* and *in vitro* methods have been proposed. *In vivo* methods using radiotracers in humans give the best estimation of bioavailability, but the use of radiotracers present problems in many laboratories (expensives or unavailables for many key trace elements); thus *in vitro* methods have been preferred for investigating trace metal bioavailability. The *in vitro* methods are usually based on the simulation of gastric and intestinal digestion of food and measure the fraction of the element available for absorption. These methods are simple, rapid, inexpensive and easy to control. Miller et al. [17] have developed a method based on element dialysability to determine iron bioavailability. This method has been used for predicting the availability from different foods. Different interlaboratory trials have been performed to evaluate the repeatability and reproducibility of these methods [18]. The pH adjustment during the intestinal digestion has been identified as one of the critical parameters in these studies. In previous papers [19,20] we have used a modification of the *in vitro* digestion method proposed by Miller et al. [17]. In the current work, we have study the use of the PIPES buffer solution proposed by Haro-Vicente et al. [21] to fill the dialysis membranes for improving the reproducibility of the *in vitro* digestion conditions. The modified *in vitro* digestion procedure was applied to edible seaweed samples to evaluate chromium, cobalt, manganese and vanadium bioavailability.

2. Materials and methods

2.1. Reagents

Ultrapure water of 18 MΩ cm obtained from a Milli-Q water-purification system (Millipore Corp., Bedford, MA, USA) was used throughout the work.

Nitric acid 69% (w/v) suprapur grade and hydrogen peroxide 33% (w/v) were used in the acid treatment of the seaweed samples and were purchased from Panreac (Barcelona, Spain). Porcine pepsin, P-7000, porcine pancreatin, P-1750, PIPES (piperazine-NN-bis(2-ethane-sulfonic acid) disodium salt and bile salts (approximately 50% sodium cholate and 50% sodium deoxycholate) (Sigma Chemicals Co., St. Louis, USA) were used for *in vitro* digestion. Hydrochloric acid (37%) analytical reagent grade from Panreac was used for preparing pepsin solution. Sodium bicarbonate, from Merck (Darmstadt, Germany) was used to prepare the intestinal solution.

NIES-09, *Sargasso* seaweed (National Institute for Environmental Studies, Ibaraki, Japan) was used as a CRM.

To avoid contamination, all glassware and storage bottles were kept in 10% nitric acid for at least 48 h. All materials were then rinsed three times with ultrapure water and preserved dried for their use.

2.2. Instrumentation

Dried seaweed was pulverized with a mortar grinder mill, model RM100 (Retsch, Haan, Germany) equipped with and agate pestle and an agate mortar. Canned seaweed in brine was freeze-dried with a LYPH-LOCK 6-L freeze-dry system, model 77530 from Labconco Corp. (Kansas City, MO, USA). To assess total elements content, seaweed samples were acid digested with an Ethos Plus microwave laboratory station (Milestone, Sorisole, Italy) inside 100-mL closed Teflon vessels with Teflon covers Model HPR 1000/10 and an HTC adapter plate and HTC safety springs (Milestone). An ORION 720A plus pH-meter with a glass-calomel electrode (ORION, Cambridge, UK) was used for pH measurements.

Total chromium, cobalt, manganese and vanadium, as well as the chromium, cobalt, manganese and vanadium in dialyzed fractions were determined by using an 820-MS inductively coupled plasma mass spectrometer (Varian, Mulgrave, Australia), equipped with an SPS3 autosampler (Varian) and a MicroMist nebulizer (Varian). A Boxcult incubator situated on a Rotabit orbital-rocking platform shaker (J.P. Selecta, Barcelona, Spain) was used to perform the enzymolysis procedure at 37 °C. Cellu Sep® H1 high grade regenerated cellulose tubular membranes (molecular weight cut-off 10 kDa, 50 cm length, diameter dry 25.5 mm and 5.10 mL cm^{-1}) were from Membrane Filtration Products Inc. (Texas, USA).

2.3. Sample preparation

Nine different types of edible seaweed harvested in the Galician coast (Northwestern Spain) were obtained from a local manufacturer. One of these samples is commercialized as cooked and canned in brine (around 25 g), and consists of a mixture of two brown seaweeds: Sea Spaghetti (*Himanthalia elongata*) and Furbelows (*Saccorhiza polyschides*). This sample was freeze-dried. The remaining samples are commercialized as dehydrated products (approximately 100 g): Dulse (*Palmaria palmata*) and Nori (*Porphyra umbilicalis*), as red seaweed; Kombu (*Laminaria ochroleuca*), Wakame (*Undaria pinnatifida*) and Sea Spaghetti (*H. elongata*), as brown seaweed; Sea Lettuce (*Ulva rigida*), as green seaweed, the microalgae *Spirulina platensis*, commonly used in human and animal nutrition; and Agar–Agar, which is a hydrocolloid obtained from the red seaweed *Gelidiumm sesquipedale*. Dehydrated samples were kept in an oven at 40 °C for 2 h to eliminate water traces before pulverization of total content of each commercialized bag in a mortar grinder mill, and were preserved in pre-cleaned polyethylene bottles.

2.4. Acid digestion procedure

Approximately 0.2 g of seaweed sample or 5 g of residue obtained after *in vitro* digestion procedure (non-dialyzable fraction) were weighed into a high pressure Teflon vessel with 8 mL of 69% (w/v) nitric acid and 2 mL of 33% (w/v) hydrogen peroxide. Closed vessels were introduced into the microwave oven. The temperature program applied had 5 steps at 1000 W of power. The initial and final temperatures were 25–90, 90–90, 90–120, 120–190, and 190–190 °C, respectively for each step. The steps 1, 3 and 4 had a ramp time of 10 min and the steps 2 and 5 a hold time of 5 and 10 min, respectively. After cooling down, the digested samples were diluted to a final volume of 25 mL with Milli-Q® water and stored in polyethylene vials at 4 °C. Acid digestion procedure was made in triplicate, and blanks were prepared as well.

2.5. In vitro digestion procedure

In vitro digestion procedure was carried out in triplicate by weighing 0.5 g of powdered seaweed into a 100 mL Erlenmeyer flask. A volume of 20 mL of ultrapure water was then added; after 15 min, the pH was adjusted to 2.0 with a 6 M hydrochloric acid solution. Then, 0.15 g of a freshly prepared gastric solution (6.0% (m/v) pepsin dissolved in 0.1 M hydrochloric acid) was added. Flasks were covered and incubated at 37 °C with orbital-horizontal shaking at 150 rpm for 120 min, and were then placed in an ice-water bath to stop the enzymatic digestion.

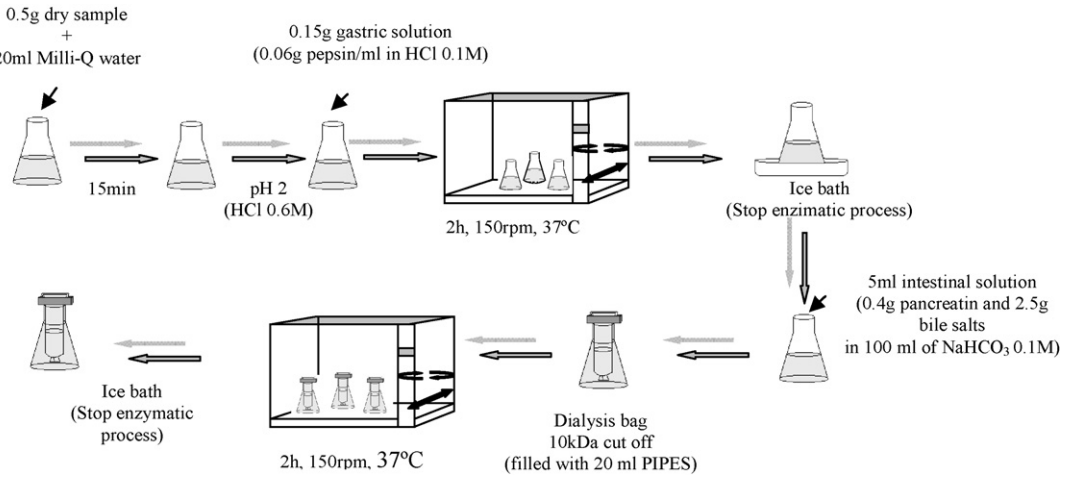

Fig. 1. *In vitro* digestion procedure.

The procedure was continuing by adding to the gastric digest. Then, 5 mL of intestinal solution (0.4% (m/v) pancreatine and 2.5% (m/v) bile salts dissolved in 0.1 M sodium hydrogen carbonate) were added to the gastric digest. At this point, dialysis membranes (10 kDa molecular weight cut-off), filled with 20 mL of a 0.15 N PIPES solution (pH 7.5 adjusted with hydrochloric acid) were placed inside the flasks. Intestinal digestion took place inside the incubator at 37 °C with an orbital-horizontal shaking at 150 rpm for 120 min. The enzymatic reaction was stopped by immersing the flasks in an ice-water bath. Dialysis membranes were removed and their outer surface was rinsed with ultrapure water. The membrane, containing the dialyzate solution, and the residual or non-dialyzable fraction, remaining in the flasks, were transferred to polyethylene vials and weighed separately. Both, dialyzated and residual fractions, were kept at −20 °C until measurements. Reagent blanks were also prepared to control possible contamination. The scheme of the *in vitro* digestion method is shown in Fig. 1.

2.6. Determination of trace elements by ICP-MS

2.6.1. Total and non-dialyzable trace elements determination by ICP-MS

Chromium, cobalt, manganese and vanadium determinations in seaweed and non-dialyzable fractions were performed by ICP-MS. The operating parameters for the instrument were as follows: rf power = 1380 W, peristaltic pump speed = 0.45 mL min^{-1}, nebulizer gas flow = 0.98 L min^{-1}, plasma gas flow = 17.0 L min^{-1}, auxiliary gas flow = 1.65 L min^{-1}. The mass to charge ratios used were ^{59}Co, ^{52}Cr, ^{55}Mn, and ^{51}V. ICP-MS determinations gave a limit of detection (LOD) and a limit of quantification (LOQ), based on the 3 SD/S and 10 SD/S criterion, respectively (S.D.: standard deviation of 11 measurements of a reagent blank; S: slope of addition curve), of 0.01 and 0.02 μg/kg for cobalt; 0.40 and 1.30 μg/kg chromium; 0.10 and 0.30 μg/kg manganese; and 0.18 and 0.62 μg/kg vanadium. Accuracy of the method was assessed by analyzing the certified reference material NIES 09 (Sargasso) in triplicate and the results obtained are shown in Table 1.

2.6.2. Trace elements determination in dialyzable fraction by ICP-MS

Chromium, cobalt, manganese and vanadium determinations in dialyzable fraction were performed by ICP-MS following the operation conditions described previously for determination of total content of these elements in seaweeds. ICP-MS determinations gave a limit of detection (LOD) and a limit of quantification (LOQ), of 0.01 and 0.03 μg/kg for cobalt; 0.71 and 2.38 μg/kg chromium; 0.13 and 0.45 μg/kg manganese; and 0.15 and 0.49 μg/kg vanadium.

3. Results and discussion

3.1. Preliminary experiments

Experiments were developed to select the enzymolisis conditions for the *in vitro* bioavailability (dialyzability) of trace elements studied from seaweed samples. These studies were performed by using a pool of different seaweed and the amount of pepsin, pancreatine and bile salts solutions recommended by Lutten et al. [18]. Three grams of pepsin solution per 10 g of food was used for gastric digestion simulation, and 0.4% (w/v) pancreatine and 2.5% (w/v) bile salts dissolved in 0.1 M sodium hydrogen carbonate were used for the intestinal digestion.

3.1.1. Comparative study of the use of PIPES buffer solution and sodium hydrogen carbonate to fill the dialysis tubes

In most of the studies related to dialyzability, including previous works developed by our research group [19,20], the dialysis membranes were filled with enough sodium hydrogen carbonate solution to obtain the physiological pH of 7.0 (equivalent to the titrable acidity as defined by the number of equivalents of sodium hydroxide required to fix the gastric digest at pH 7.0) [17]. This procedure presents the following drawbacks: first, the pH can vary during the intestinal digestion process, and second, an acid–base titration is necessary to know the required amount of sodium hydrogen carbonate.

An alternative method proposed by Haro-Vicente et al. [21] is the use of PIPES buffer solution instead of sodium hydrogen carbonate solution to fill the dialysis tubes. This buffer solution was used to maintain a physiological pH. These authors concluded that the pH of the PIPES solution inside the membranes is constant along the intestinal digestion process, and it is not necessary to perform

Table 1
Accuracy of the method for Co, Cr, Mn and V determination in seaweed by ICP-MS using NIES 09 certified reference material (Sargasso).

Element	Certified value (μg/g)	Obtained value ($n = 3$) (μg/g)
Co	0.12 ± 0.01	0.10 ± 0.020
Cr	0.2[a]	2.4 ± 0.30
Mn	21.2 ± 1.0	17.2 ± 1.10
V	1.0 ± 0.1	1.1 ± 0.10

[a] Indicative value.

Table 2
pH measurements in the dialyzate and non-dializable fraction, using PIPES and Na_2CO_3 to fill the dialysis bag.

Dialysis bag content	Initial pH	Final pH[a]	
		pH dialyzate	pH residual fraction
0.15N PIPES	6.4	6.3 ± 0.0089	6.1 ± 0.15
0.15N PIPES	6.9	6.8 ± 0.0060	6.6 ± 0.11
0.15N PIPES	7.5	7.3 ± 0.017	6.9 ± 0.072
0.15N PIPES	7.85	7.6 ± 0.045	6.9 ± 0.10
0.15N PIPES	8.5	7.8 ± 0.041	7.1 ± 0.025
0.035N Na_2CO_3	7.0	8.5 ± 0.31	7.8 ± 0.50

[a] $n = 2$.

an acid–base titration of the gastric digestion to know the exact amount of PIPES inside the dialysis membranes.

Therefore, experiments were developed in this work to compare the pH (fixed between 6.4 and 8.5) by using a 0.15 N PIPES solution and 0.035 N sodium hydrogen carbonate solution (pH 7.0). Each experiment was performed in duplicate following the description given in Section 2.5. The results obtained in this experiment are shown in Table 2. It can be seen that higher deviations from the fixed initial pH have been found for the use of the sodium hydrogen carbonate solution. When PIPES buffer solution was used, pH measurements in dialyzate and residual fractions were close to initial pH (7.5). Thus, PIPES at pH 7.5 was selected because the pH of both dialyzate and residual fractions were close to the initial pH (7.31 ± 0.017 and 6.94 ± 0.072, respectively).

3.1.2. Influence of the molecular weight cut-off for dialysis membranes

Three molecular weight cut-off membranes (MWCO) (1, 5 and 10 kDa) were studied to compare the effect of the MWCO on the dialyzability of Co, Cr, Mn and V from seaweed. Each experiment was performed in duplicate and the analysis of reagents blanks gave a negligible concentration after ICP-MS analysis of dialyzates. Concentration of trace elements in the dialyzate fraction, referred to the initial amount of seaweed, are shown in Table 3. The highest dialyzability value was obtained with a 10 kDa MWCO; therefore, this MWCO was selected to perform this study.

3.2. Mass-balance study

In order to assess the accuracy of the analysis method, a mass-balance approach was performed by using the seaweed sample Kombu. After the *in vitro* procedure (Section 2.5), Co, Cr and Mn concentrations were determined in the dialyzates (Section 2.6.2), similarly these elements were determined in the residual fractions (Section 2.6.1) after acid digestion (Section 2.4). The *in vitro* digestion as well as the microwave acid digestion of the seaweed sample and the residual fractions, were performed in triplicate. Results obtained are shown in Table 4. After analysis, the sum of the Co, Cr and Mn concentrations in both fractions (dialyzable and non-dialyzable) was statistically compared with the total Co, Cr and Mn concentrations in the seaweed sample. First, a statistical comparison of the standard deviation was established by means of the Cochran's C and Bartlett's tests. The *p*-values after Cochran's C and Bartlett's test at a 95.0% confidence level ($\alpha = 0.05$) are respectively 0.1165 and 0.1120 for Co, 0.7533 and0.7850 for Cr, and 0.1305 and 0.1273 for Mn. As it can be seen, the smallest of the *p*-values is higher than 0.05 (95% confidence level) for cobalt, chromium and manganese. This indicates that there is no statistically significant difference among the S.D., and an ANOVA test can be performed to compare means. The *p*-values are higher than 0.05 (95% confidence interval) for each of the elements studied (Co-0.3508, Cr-0.3900, Mn-5.32) which means that there are no statistically significant differences between the total element concentration, and the sum of element concentration in the dialyzate and the non-dialyzable fraction.

Table 3
Influence of the molecular weight cut-off for dialysis membranes in the element content (concentration (μg/g) ± S.D.) in dialyzable fraction.

Element	1 kDa	5 kDa	10 kDa
Co	0.04 ± 0.005	0.06 ± 0.002	0.06 ± 0.005
Cr	0.02 ± 0.02	0.10 ± 0.0059	0.14 ± 0.012
Mn	6.69 ± 0.608	8.67 ± 0.505	9.70 ± 0.744
V	3.35 ± 0.365	4.22 ± 0.591	4.96 ± 0.399

Table 4
Mass-balance study.

Element	Total (μg/g)	Dialisate + residue (μg/g)
Co	0.12 ± 0.040	0.14 ± 0.010
Cr	6.8 ± 0.51	6.6 ± 0.61
Mn	5.3 ± 1.4	6.7 ± 0.56

V is not included because the total content in the sample studied is less than LOQ.

3.3. Study of Co, Cr, Mn and V bioavailability in different types of seaweed samples

The determination of total Co, Cr, Mn and V concentration in the different types of edible seaweed samples studied has been performed using the proposed methods. In the first step of this study, the total content of each sample bag or can were homogenized (Section 2.3). The samples were acid digested (Section 2.4), and analyzed in triplicate (Section 2.6.1), and the results obtained are shown in Table 5. The cobalt content in the seaweeds studied is between 1.2 and 0.08 μg/g for Sea Spaghetti and Agar–Agar, respectively. The levels of chromium are higher than cobalt levels and are between 6.8 μg/g (Kombu and canned seaweed) and 1.9 μg/g (Nori). For manganese and vanadium the variability of total element content is higher than cobalt and chromium. Manganese total content is between 233.1 μg/g for Dulse and 5.3 μg/g for Kombu. Vanadium content in the seaweed samples studied varies from 130.1 μg/g (Dulse) to levels lower than the detection limit of the determination method (0.18 μg/kg) for Kombu and Agar–Agar.

Moreover, a study of the bioavailability in the same seaweed samples was performed using the proposed *in vitro* digestion procedure (Section 2.5). The results obtained for Co, Cr, Mn and V in the dialyzable fraction are shown in Table 6. Cobalt content in dialyzable fraction of seaweed samples after the *in vitro* digestion procedure was between 0.68 μg/g (Sea Spaghetti) and lower than the detection limit (0.01 μg/kg) for Nori, Agar–Agar and Spirulina. Chromium levels in the dialyzable fraction were between 4.2 μg/g for Wakame and lower than the detection limit (0.71 μg/kg) for Nori, Agar–Agar and Spirulina. Manganese levels in the dialyzable fraction are higher than the levels of the other elements studied in the same samples, and they vary between 214.1 μg/g (Dulse) and 1.2 μg/g (Agar–Agar). Vanadium was not detected (levels lower than 0.15 μg/kg) in Nori, canned seaweed, Agar–Agar and Spirulina, and the levels in the other samples vary from 78.8 μg/g (Dulse) to 1.1 μg/g (Kombu). In this study, the bioavailability of the trace elements studied is estimated as the dialyzability, which was calculated dividing the dialyzable fraction by the total element content multiplied by 100 (Fig. 2). Manganese is the element with the highest value of dialyzability, independently of the type of seaweed sample. In function of the type of seaweed, the highest dialyzability percentages were found in Wakame (77.5 ± 0.22% Co, 66.7 ± 0.24% Cr, 100.0 ± 0.03% Mn). The total vanadium content found in Wakame was lower than the limit of quantification (LOQ). This is the reason we have no result for the dialyzability percentage

Table 5
Total content of trace elements in seaweed samples ($n = 3$) by ICP-MS.

Seawed sample	[Co] (μg/g)	[Cr] (μg/g)	[Mn] (μg/g)	[V] (μg/g)
Wakame (*Undaria pinnatifida*)	0.36 ± 0.010	6.3 ± 0.30	6.8 ± 0.11	<LOQ[a]
Kombu (*Laminaria ocholeuca*, *Laminaria sacharina*)	0.12 ± 0.040	6.8 ± 0.49	5.3 ± 1.4	<LOD[a]
Dulse (*Palmaria elongata*)	0.35 ± 0.040	6.6 ± 0.60	233.1 ± 6.60	130.1 ± 11.51
Sea Spaghetti (*Himanthaliae elongata*)	1.2 ± 0.040	3.2 ± 0.40	46.8 ± 1.31	15.4 ± 0.491
Sea Lettuce (*Ulva rigida*)	0.63 ± 0.010	6.4 ± 0.11	91.4 ± 0.810	6.2 ± 0.20
Nori (*Porphyra umbilicales*, *Porphyra linearis*)	0.23 ± 0.030	1.9 ± 0.10	27.0 ± 2.8	1.4 ± 0.20
Canned seaweeds (cooked *Himanthalia elongata* and *Saccorhiza polyschides*)	1.02 ± 0.0380	6.8 ± 0.59	34.7 ± 0.910	7.9 ± 0.70
Agar–Agar (*Gelidium Sesquipedale*)	0.08 ± 0.01	2.4 ± 0.20	10.0 ± 1.3	<LOD[a]
Spirulina (Spirulina plantesis)	0.33 ± 0.018	2.3 ± 0.20	29.5 ± 0.820	0.7 ± 0.03

[a] LOD = 0.18 μgV/kg, LOQ = 0.62 μgV/kg.

Table 6
Content of trace elements in dialyzable fraction of seaweed samples ($n = 6$) by ICP-MS.

Seawed sample	[Co] (μg/g)	[Cr] (μg/g)	[Mn] (μg/g)	[V] (μg/g)
Wakame (*Undaria pinnatifida*)	0.28 ± 0.05	4.2 ± 0.8	6.8 ± 1.6	1.3 ± 0.3
Kombu (*Laminaria ocholeuca*, *Laminaria sacharina*)	0.04 ± 0.004	2.1 ± 0.3	3.3 ± 0.4	1.1 ± 0.2
Dulse (*Palmaria palmata*)	0.18 ± 0.03	2.1 ± 0.3	214.1 ± 13.7	78.8 ± 7.24
Sea Spaghetti (*Himanthalia elongata*)	0.68 ± 0.07	1.9 ± 0.5	36.2 ± 3.4	4.5 ± 0.8
Sea Lettuce (*Ulva rigida*)	0.22 ± 0.05	1.9 ± 0.8	65.0 ± 18.0	2.5 ± 0.6
Nori (*Porphyra umbilicales*, *Porphyra linearis*)	<LOD[a]	<LOD[a]	18.9 ± 6.6	<LOD[a]
Canned seaweeds (cooked *Himanthalia elongata*, *Saccorhiza polyschides*)	0.05 ± 0.02	0.6 ± 0.3	6.3 ± 1.2	<LOD[a]
Agar–Agar (*Gelidium Sesquipedale*)	<LOD[a]	<LOD[a]	1.2 ± 0.2	<LOD[a]
Spirulina (*Spirulina plantesis*)	<LOD[a]	<LOD[a]	4.6 ± 0.7	<LOD[a]

[a] LOD: 0.01 μgCo/kg; 0.71 μgCr/kg; 0.15 μgV/kg.

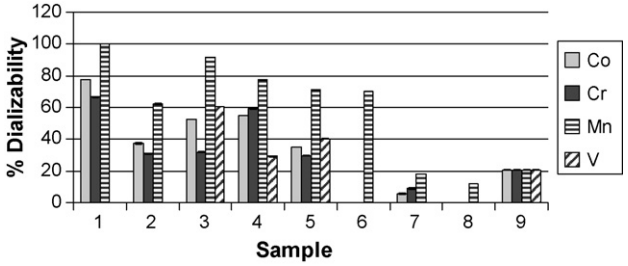

Fig. 2. Dialyzability of cobalt, chromium, manganese and vanadium in seaweed samples. (1) Wakame, (2) Kombu, (3) Dulse, (4) Sea Espaghetti, (5) Sea Lettuce, (6) Nori, (7) Canned seaweed, (8) Agar–Agar, (9) Spirulina.

of vanadium in this sample. The lowest dialyzability percentages were obtained for Agar–Agar and cooked canned seaweed, but this is because cobalt, chromium and vanadium were no detected in the dialyzable fraction. The results obtained for the Spirulina (Fig. 2) are very interesting: the percentage of dialyzability was very similar for all the elements studied (20.6 ± 0.47%) meanwhile in the rest of the samples this value varies in function of the element studied. This difference probably it is due because spirulina it is a microscopical seaweed.

4. Conclusions

An *in vitro* digestion method for assessing the bioavailability of chromium, cobalt, manganese and vanadium in seaweed samples, using PIPES as a buffer solution and dialysis membranes, has been evaluated. An accurate and sensitive method (ICP-MS) was used to determine the trace element concentrations in the different fractions (total in acid digested samples, dialyzable and non-dialyzable fractions). The proposed method was applied to study the cobalt, chromium, manganese and vanadium bioavailability in different types of samples (red, brown and green seaweeds), obtaining dialyzability percentages between 0 and 100%. The highest dialyzability percentages were found for manganese in all the samples studied. We have not found literature for evaluating cobalt, chromium, manganese and vanadium bioavailability in seaweed to compare with our results; only the determination of total content of these elements in different seaweeds is published. Taking into account the importance of seaweed in human nutrition, as a food or as an additive of processed food, more studies regarding bioavailability are needed for assessing the safety and knowing the real nutritional value of this foodstuff. The in vitro digestion procedures are a good, inexpensive and adequate way to manage to make it.

It is difficult to explain the results obtained for the different types of seaweed. In order to explain these differences, it is necessary to have more information about the different chemical species present in seaweed and in the dialyzate. This is the objective of our next study.

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