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ORIGINAL PAPER

Determination of perchlorate and iodide concentrations in edible seaweeds

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Abstract Perchlorate and iodide concentrations were determined in brown (*Undaria pinnatifida* and *Laminaria japonica*) and red (*Porphyra* sp.) edible seaweeds, which are commonly consumed by Korean people, with the use of ion chromatography, coupled with a tandem mass spectrometer. Seaweeds (i.e., good sources of iodine) are among the most important plant life in the ocean and commonly consumed as food and nutritional supplement in South Korea. All seaweed samples were purchased from different regions in South Korea. The detected concentrations of perchlorate were as follows: 19.7–620.7 μg kg^{-1} dry weight ($n = 11$, mean concentration = 149.2 μg kg^{-1} dry weight) for *L. japonica* and 7.3–21.7 μg kg^{-1} dry weight (mean concentration = 10.6 μg kg^{-1} dry weight) for *U. pinnatifida*. Of the 11 samples of *Porphyra* sp., only 1 sample showed 6.7 μg kg^{-1} dry weight perchlorate. The concentrations of iodide in all seaweed samples varied from 0.44 to 6,800 mg kg^{-1} dry weight. *L. japonica* samples ($n = 11$) had significantly higher iodide concentrations, with a mean of 5,261 mg kg^{-1} dry weight. The bioconcentration factor values for perchlorate and iodide in the three different seaweeds varied widely and showed similar variation trends. The trend for perchlorate and iodide was *Porphyra* sp. < *U. pinnatifida* < *L. japonica*. The results have provided growing evidence that perchlorate frequently occurs in food products.

Keywords *Laminaria japonica* · Occurrence · *Porphyra* sp. · *Undaria pinnatifida*

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Introduction

Edible seaweeds are commonly consumed in many countries, notably Korea, Japan, and China. Over 6 million tons of fresh seaweed varieties are now cultivated per year worldwide (FAO 2011). In Asian countries, seaweeds have been used as a traditional source of food since prehistoric times; red and brown seaweeds are mainly employed as food sources (Chapman and Chapman 1980). In Western countries, seaweeds have been used for the extraction of iodide; additionally, fresh seaweeds have been used as a source of food (Briand 1991). With the current trend in the consumption of healthy foods, there has been an increase in the direct consumption of seaweeds because they provide nutritional benefits and have a number of medicinal properties (Mabeau and Fleurence 1993).

Seaweeds are known to be the richest source of iodine, which is an essential nutrient for human health. The most common forms of natural iodine in edible seaweed products are predominantly iodide, with iodate and iodized organic compounds providing a small fraction of bioavailable iodine (Shah et al. 2005). A previous study has reported that the percentage values of each bioavailable

species for different seaweeds including Wakame (*Undaria pinnatifida*), Kombu (*Laminaria japonica*), and Nori (*Porphyra* sp.) were 3.0 ± 1.0, 18.2 ± 2.0, and 5.0 ± 1.0, respectively. Lack of iodine has a negative influence on thyroid hormone production and results in developmental diseases, neurological damage, goiter, and paralysis (de Benoist et al. 2004), while excessive iodine dietary intake can lead to serious pathological problems. Hence, it has been established that the recommended dietary allowance for iodine ranges from 140 to 160 μg day^{-1} for adults (Recommended Nutrient Intakes for Canadians 1983; Recommended Dietary Allowances 1989; Dietary Reference Values for Food 1991).

Perchlorate is widely known to be an inorganic endocrine disruptor because it influences the thyroid gland by competitively inhibiting iodide transport (Snyder et al. 2003). While there is no federal regulation in the USA and South Korea, perchlorate is a regulated drinking water contaminant in California in the USA with a maximum contaminant level of 6 mg L^{-1} (CDPH 2012). In South Korea, a study detailed the occurrence of perchlorate at various concentrations ranging from 0.05 to 60 μg L^{-1} in surface water, 0.56 to 22 μg L^{-1} in wastewater treatment plant effluent, and 0.2 to 35 μg L^{-1} in tap water (Quinones et al. 2007). In a separate study, perchlorate detection data in South Korea was presented as follows: 0.10–6.10 μg L^{-1} for tap water ($n = 520$), 0.04–0.29 μg L^{-1} for bottled water ($n = 48$), and 0.11–6.11 μg L^{-1} for seawater ($n = 9$) (Her et al. 2011). These studies suggest that perchlorate is present not only in drinking water sources, but also in seawater in South Korea.

Numerous recent studies have reported that perchlorate can also be detected in vegetable samples and terrestrial plants, confirming that most of the perchlorate has a tendency to accumulate in leafy tissue rather than in other parts of a plant (Hutchinson 2003; Yu et al. 2004; Seyfferth and Parker 2007). It was suggested that transpiration is the major mechanism for the transport of perchlorate from the root to the top of the plants (Yu et al. 2004; Seyfferth and Parker 2007). However, there is little information on the capacity of perchlorate to accumulate in seaweeds, the relation between the accumulation of iodide and that of perchlorate in seaweeds, and the mechanism by which perchlorate accumulation occurs.

In view of the current increasing demand for seaweed products and the paucity of information regarding perchlorate in edible seaweeds, the aim of this work was to investigate the concentrations of perchlorate and iodide present in 3 different seaweed samples (*Undaria pinnatifida*, *Laminaria japonica*, and *Porphyra* sp.) growing near the coast of South Korea. The authors also aimed to provide information on the bioconcentration factors (BCFs) of perchlorate and iodide in these seaweed products by investigating the concentrations of perchlorate and iodide in a limited number of seawater samples. To the best of the authors' knowledge, this is the first comprehensive study focusing on the assessment of perchlorate levels in seaweed products in South Korea. This study was performed from August 2009 through December 2010 in South Korea.

Materials and methods

Materials

All solutions and dilutions were prepared using ultrapure water purified with the MQ water purification system (Milli-Q; Millipore, Molsheim, France). A perchlorate standard solution of 1,000 mg L^{-1} purchased from Accustandard Inc. (New Haven, CT, USA) was used for the calibration run. ^{18}O-enriched $NaClO_4$ (Icon Services Inc., NJ, USA) was used as an internal standard for perchlorate analysis to provide accurate quantification by compensating for matrix effects. Glacial acetic acid and acetonitrile, all of which were of analytical reagent grade, were purchased from JT Baker, Phillipsburg, NJ, USA. Glacial acetic acid was diluted with distilled water to form a 1 % (v/v) solution for use in the extraction steps.

Seaweed collection and preparation

Brown seaweeds, *U. pinnatifida* (Wakame) and *L. japonica* (Kombu), and red seaweed, *Porphyra* sp. (Nori), were used to determine perchlorate levels. From August 2010 to December 2010, 30 seaweed samples, which were harvested from 3 different coasts of South Korea, were purchased from local markets in 7 provinces. All samples were commercially available as dehydrated products. Because of their hygroscopic nature, all seaweed samples were ground and then stored in air-tight containers until pre-analytic treatment. Figure 1 describes the sampling locations or manufacture locations for three different seaweed samples.

To determine the levels of perchlorate and iodine in the seaweed samples, 3 g of the ground sample was placed in a 50-mL polypropylene conical tube and supplemented with 1.6 mL of the 300 μg L^{-1} $NaCl^{18}O_4$ solution to function as a 12 μg L^{-1} internal standard. After 20 mL of 1 % acetic acid and 20 mL of can were added, the tightly capped tube was vortexed for 1 min, and then shaken using a mechanical shaker (SHO-2D; WiseShake, Daihan Scientific Co. Ltd., Seoul, Korea) at a speed of 250 rpm for 10 min at room temperature. The mixture was then centrifuged at 4,000 rpm for 20 min, and the supernatant was passed through a preconditioned Supelclean Envi-Carb SPE cartridge and filtered through a 0.2-μm pore size syringe filter for IC-MS/MS analysis.

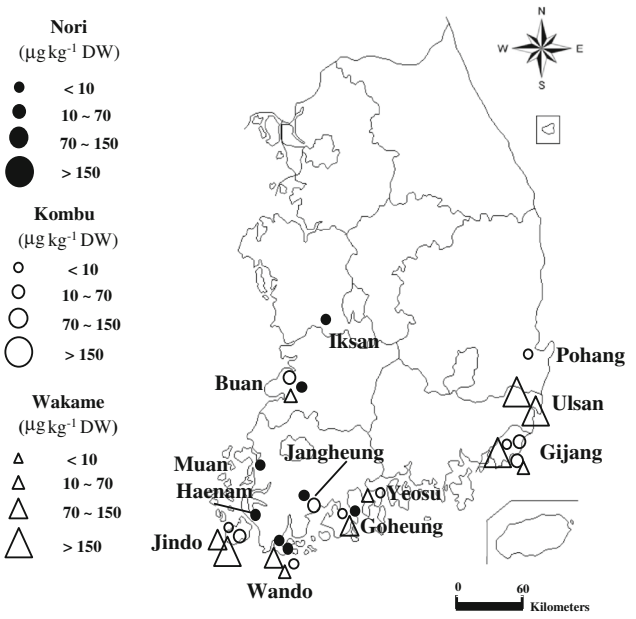

Fig. 1 Sampling locations and distribution of perchlorate average concentration in edible seaweeds

Seawater collection and preparation

Seawater samples were collected from 18 different locations (1 sample from each location) from August 2009 to August 2010. All seawater samples were collected within 50 m from the shore during high tide and were undoubtedly affected by coastal runoff. The samples were then stored at 4 °C until pre-analytic treatment.

To determine the levels of perchlorate in the seawater samples, 6 mL of each seawater sample was treated in series, using OnGuard Ba, OnGuard Ag, and OnGuard H cartridges to remove the chloride and sulfate ions in the seawater, and then filtered with a 0.2-μm pore size syringe filter.

The iodide content was only determined for the seawater samples ($n = 4$) collected from the south coast of South Korea. Because of the high ionic strength matrix, due to high chloride, carbonate, and sulfate concentrations, direct analysis of the iodide concentrations in the seawater samples was difficult. Therefore, these seawater samples were not treated with the OnGuard Ag cartridge because iodide can be removed at the same time as chloride. After being diluted 50 times with ultrapure water, each sample was filtered with a 0.2-μm pore size syringe filter for IC-MS/MS analysis.

Instrumental analysis

The seaweed extracts and the seawater samples were analyzed using IC-MS/MS (Dionex ICS 2100 and Agilent 6410 triple quadrupole mass spectrometers) method. A thorough description of the analytical methods utilized has been published previously (Her et al. 2010, 2011). For the detection of perchlorate, the calibration standards and the seaweed extracts were all supplemented with $NaCl^{18}O_4$ solution as a 12 μg L^{-1} internal standard. Perchlorate was quantified using the ratio of perchlorate to the internal isotopic perchlorate standard. The method detection limit (MDL) was calculated by multiplying the standard deviation (SD) of the replicate measurements; the limit of quantification (LOQ) was then calculated by multiplying MDL by 7. For iodide, external standard calibration was used.

Table 1 Recoveries of perchlorate anion fortified in the three different seaweed species

Species (common name)	Fortification level (μg kg^{-1})	Average % recovery ($n = 3$)	R.S.D (%)
Porphyra sp. (Nori)	10.0	98.2	12.0
	20.0	104.7	16.3
Undaria pinnatifida (Wakame)	10.0	104.3	9.8
	20.0	109.8	11.5
Laminaria japonica (Kombu)	10.0	103.8	3.4
	20.0	101.6	4.4

The instrument (IC-MS/MS) was calibrated with perchlorate standards prepared in MQ water at concentrations ranging from 0.5 to 50 μg L^{-1}. Correlation coefficients for all the calibrated analytes typically exceeded 0.997. The MDL for perchlorate in the seaweed samples was 0.07 μg kg^{-1}, and the LOQ was 0.52 μg kg^{-1}. A fortifying test was conducted to evaluate the accuracy and precision of the method, which reported the mean percent recovery and the relative standard deviation (RSD), respectively. The results for the perchlorate levels of *U. pinnatifida*, *L. japonica*, and *Porphyra* sp. samples fortified with 10 and 20 μg kg^{-1} are presented in Table 1. Precision (%RSD) values ranged from 3.4 to 16.3 %, suggesting good reproducibility of this method. Compared to the theoretical concentration used to determine the recovery, the calculated concentrations were generally between 98.2 and 109.8 %, indicating that the method was sufficiently accurate for the analysis of perchlorate in seaweeds.

Results and discussion

Perchlorate in seaweed samples

The perchlorate concentrations, with standard deviations for each of the seaweed samples, which were cultivated from three different coasts of South Korea, are reported in Table 2. In addition, the results comparing the three

different seaweeds are shown in Fig. 1. The perchlorate concentration in the seaweed samples varies from non-detection to 620.7 μg kg^{-1}. *Porphyra* sp. (Nori) samples have the lowest concentrations, followed by *U. pinnatifida* (Wakame) and *L. japonica* (Kombu). This perchlorate content gradation is related to the type of seaweed (Kombu > Wakame > Nori) and has been described previously (Martinelango et al. 2006). The perchlorate concentrations in *L. japonica* were particularly high, ranging from 19.7 to 620.7 μg kg^{-1} dry weight (DW), with an average concentration of 149.2 μg kg^{-1} DW. The highest perchlorate value of 620.7 μg kg^{-1} DW was detected in the sample from Jindo, although the concentrations of perchlorate in the *U. pinnatifida* samples were generally lower than those found in the *L. japonica* samples, with a mean concentration of 10.6 μg kg^{-1} DW, the perchlorate levels were detected at different concentrations in all the samples (7.3–21.7 μg kg^{-1} DW). Of the 8 sites where *Porphyra* sp. (Nori) was sampled, only 1 sample (6.7 μg kg^{-1} DW) exceeded the LOQ.

Although the determination of perchlorate in the processed seaweeds purchased from local markets has been reported in this study, the results of this study may be different from those reported by other authors who directly collect seaweed samples on the coast. Because the seaweed species used in this study were subject to a washing process to remove dust and some solid particles during the entire manufacturing process, perchlorate could have been washed off the seaweed samples. A separate study demonstrates that up to 70 % of perchlorate is removed from seaweed samples by rinsing them once in deionized water, suggesting that one of the mechanisms for accumulation of perchlorate might be adsorption, including precipitation and exchangeable sorption on the surface (Martinelango et al. 2006). In addition, farmers treat *Porphyra* sp. using a type of acid—called "sea laver treatment acid"—during the cultivation season to improve the production efficiency and the quality of the seaweed. This treatment practice also has a huge influence on the removal of perchlorate in *Porphyra* sp.

Iodide in seaweed samples

Figure 2 shows a graphical representation comparing the results of the iodide concentrations for three different seaweeds. *Porphyra* sp. samples ($n = 8$) had a range of 0.44–3.59 mg kg^{-1} DW iodide with a mean ± standard deviation of 1.36 ± 0.99 mg kg^{-1} DW. For *U. pinnatifida*, iodide was detected in all samples ($n = 11$) at higher concentrations than that in *Porphyra* sp. and at levels ranging from 4.56 to 34.58 mg kg^{-1} DW with a mean of 13.60 ± 8.64 mg kg^{-1} DW. *Laminaria* species are known to be effective iodine accumulators (Kupper et al. 1998, 2008). In this study, *L. japonica* samples ($n = 11$) had

Table 2 Perchlorate concentration in the three different seaweed species and seawater

Species (common name)	Perchlorate concentration (μg kg^{-1} DW ± SD)		
	The south coast of Korea	The east coast of Korea	The west coast of Korea
Porphyra sp. (Nori, $n = 8$)	ND[a]	NA[b]	ND[a]
	ND[a]	NA[b]	ND[a]
	ND[a]	NA[b]	6.7 ± 0.2
	ND[a]	NA[b]	ND[a]
Undaria pinnatifida (Wakame, $n = 11$)	7.9 ± 0.6	11.7 ± 0.5	10.0 ± 0.9
	8.7 ± 1.3	21.7 ± 2.7	8.5 ± 0.4
	8.3 ± 0.3	8.1 ± 1.7	7.3 ± 0.2
	17.2 ± 0.4	7.4 ± 0.8	NA[b]
Laminaria japonica (Kombu, $n = 11$)	71.8 ± 4.1	223.0 ± 42.4	620.7 ± 70.8
	57.3 ± 2.8	154.3 ± 3.3	109.3 ± 5.8
	119.7 ± 4.7	174.6 ± 5.8	44.6 ± 0.9
	46.6 ± 0.1	19.7 ± 0.8	NA[b]
Location	Average conc. of ClO_4^- (μg L^{-1})[c]	S.D.	
The west coast of South Korea ($n = 2$)	0.24	0.05	
The south coast of South Korea ($n = 5$)	0.33	0.10	
The east coast of South Korea ($n = 4$)	0.28	0.16	

[a] Not detected

[b] Not collected. In the east coast of Korea, *Porphyra* sp. was not cultivated because of the heavy waves. All values are means of triplicate extractions ± SD

[c] LOQ is 0.03 μg L^{-1}, duplicate samples were analyzed in all cases

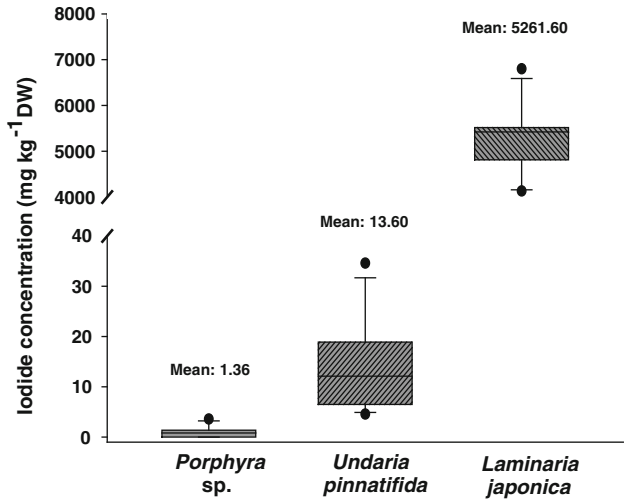

Fig. 2 Box-and-whisker plot of iodide concentration in the three different seaweed species

significantly higher iodide concentrations ranging from 4,129 to 6,800 mg kg^{-1} DW. The results show the interrelationship of perchlorate versus iodide rate. In all the seaweed species studies, perchlorate and iodide concentrations have good positive correlations. This suggests that, in these seaweeds, the perchlorate may be taken up in a similar fashion as iodide.

Perchlorate and iodide concentrations in seawater

The results comparing the average perchlorate concentrations in three different coast seawater samples are shown in Table 2. For each of the seawater samples, the MDL was 0.013 μg L^{-1} and the LOQ was 0.032 μg L^{-1} for IC-MS/MS. Perchlorate was detected in all seawater samples, which were higher than the LOQ, and had a range from 0.11 to 0.45 μg L^{-1} with a mean of 0.29 ± 0.12 μg L^{-1}.

Seawater is a large natural source of iodide, generally containing 50–60 μg L^{-1} iodide, but the concentration range is quite wide. However, iodide levels can be detected from less than 1 μg L^{-1} to more than 60 μg L^{-1}, depending on water depth, oxygen concentration, and biological mediation of the iodide/iodate equilibrium (Tian and Nicolas 1995). In this study, the iodide concentrations in the seawater samples from the south coast were 21.7–26.3 μg L^{-1} with a mean of 24.3 ± 1.8 μg L^{-1}. The results are unclear how perchlorate and iodide concentrations in seawater correlate with the respective concentrations found in the seaweeds. However, more extensive research is needed in the future to determine the source and fate of perchlorate and iodide in food and water.

Bioconcentration factor

For each of the three different species of seaweeds, the BCFs of perchlorate and iodide were calculated as the ratio of the mean perchlorate and iodide concentrations in the seaweeds to their mean concentrations in the seawater used in this study. The BCFs reflect the capacity of seaweeds to accumulate perchlorate and iodide from seawater. Because the values of the BCFs are dependent on the individual seaweed and seawater elemental concentration, the efficiency of the BCFs is better understood when compared between different harvest intervals, seaweed species, and elements.

The BCFs for the three different species of seaweeds for perchlorate (BCF_p) and iodide (BCF_i) are reported in Table 3. It is interesting to note that the mean iodide concentration in *L. japonica* was approximately 200,000 times higher than the value in the seawater samples. The accumulated perchlorate amount was about 500-fold from seawater, while *Porphyra* sp. accumulated the smallest amount of iodide and perchlorate among the three different seaweed species. In addition, the BCF values for perchlorate and iodide in the three different seaweeds showed similar variation trends. The trend with regard to perchlorate and iodide was *Porphyra* sp. < *U. pinnatifida* < *L. japonica*.

The ratio of BCF_i to BCF_p was also calculated to compare the accumulation trend by which the three different seaweeds accumulate perchlorate (Table 3). The values varied over 2.5 orders of magnitude, ranging from 3.1 to 964.7. The results suggest that *Porphyra* sp. may concentrate perchlorate using a similar mechanism to iodide, whereas *U. pinnatifida* and *L. japonica* accumulate iodide more selectively compared to perchlorate. The findings also suggest that the concentration of perchlorate

Table 3 Bioconcentration factors of perchlorate and iodide for the three different seaweed species

Species	BCF_p	BCF_i	BCF_i/BCF_p
Porphyra sp. ($n = 8$)	23.1 ± 0.4[a]	56.0 ± 40.6	3.1 ± 2.7
Undaria pinnatifida ($n = 11$)	36.6 ± 16.0	559.8 ± 355.7	16.8 ± 14.0
Laminaria japonica ($n = 11$)	507.8 ± 573.0	216,674 ± 30154.2	964.7 ± 924.1

Perchlorate concentration was detected in only one sample

[a] $n = 1$

and iodide by these seaweeds unlikely involves simple anion exchange mechanisms, since perchlorate is all the time more selectively up-taken relative to iodide. Therefore, it is assumed that iodide is additionally taken up by independent redox-mediated processes, while both perchlorate and iodide are taken up by ion exchange processes (Martinelango et al. 2006).

Conclusion

In this work, the concentrations of both perchlorate and iodide in three different seaweed species (*Porphyra* sp., *U. pinnatifida*, and *L. japonica*) sold in South Korea were investigated using IC-MS/MS to detect their levels. The perchlorate and iodide contents were found to vary over a wide range for the three different seaweed species—iodide ranging from 0.44 to 6,800 mg kg^{-1} DW and perchlorate from ND to 620.7 μg kg^{-1} DW. The order of the mean perchlorate and iodide concentrations determined in this study for the three different seaweed species was *Porphyra* sp. < *U. pinnatifida* < *L. japonica*. The BCF results indicate that the mean perchlorate concentration in *L. japonica* samples was approximately 500 times higher than the amount found in seawater samples. This study suggests that potential per capita dietary exposure to perchlorate and iodide in seaweeds can be estimated for Korean population and selected subpopulations by multiplying the average seaweed intake (g kg^{-1} body weight per day) by the perchlorate and iodide concentrations in the seaweed consumed.

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