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Analysis of iodine content in seaweed by GC-ECD and estimation of iodine intake



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ABSTRACT

Edible seaweed products have been consumed in many Asian countries. Edible seaweeds accumulate iodine from seawater, and are therefore a good dietary source of iodine. An adequate consumption of seaweed can eliminate iodine deficiency disorders, but excessive iodine intake is not good for health. The recommended dietary reference intake of 0.15 mg/d and 0.14 mg/d for iodine has been established in the United States and Taiwan, respectively. In this study, 30 samples of seaweed were surveyed for iodine content. The samples included 10 nori (*Porphyra*), 10 wakame (*Undaria*), and 10 kombu (*Laminaria*) products. The iodine in seaweed was derivatized with 3-pentanone and detected by gas chromatography-electron capture detector (GC-ECD). The method detection limit was 0.5 mg/kg. The iodine content surveyed for nori was 29.3–45.8 mg/kg, for wakame 93.9–185.1 mg/kg, and for kombu 241–4921 mg/kg. Kombu has the highest average iodine content 2523.5 mg/kg, followed by wakame (139.7 mg/kg) and nori (36.9 mg/kg). The GC-ECD method developed in this study is a low-cost alternative to inductively coupled plasma-optical emission spectroscopy for iodine detection in seaweeds. The iodine intake from seaweed in the current survey was calculated and compared with the iodine dietary reference intake of Taiwan. The risk and benefit of seaweed consumption is also discussed.

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1. Introduction

Marine algae are rich in dietary fiber, vitamins, and minerals, as well as long-chain polyunsaturated fatty acids [1–5]. Because seaweeds are low in calories and full of nutrients, adequate consumption of seaweeds is beneficial to health [6–8]. Marine algae nori (*Porphyra*), wakame (*Undaria*), and kombu (*Laminaria*) are popular food ingredients in Asian countries such as Taiwan, China, Japan, and Korea. Seaweeds

are well-known food sources with rich iodine content [9]. Iodine is required throughout life and is related to proper cognition function development for children [10]. Although iodine is essential for proper thyroid function, too much or too little iodine is harmful to health. In recent years, reports of food recall due to excessive iodine content in Australia, Ireland, Singapore, and the European Commission Rapid Alert System for Food and Feed have raised concerns internationally, especially for seaweed and seaweed products. Iodine-induced toxic

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effects through consumption of kelp-containing tea have been reported by Müssig et al [11]. Crawford et al [12] reported serious thyroid dysfunction in those who consume soy milks manufactured with kombu in Australia, and these soy milk products were recalled in Australia, Ireland, and Singapore. A very high iodine content of 22,911 mg/kg in dried seaweed has been reported by the European Commission Rapid Alert System for Food and Feed [13]. However, occasionally food with low iodine content will also cause consumer concern. According to the news released from the Center for Food Safety in Hong Kong, some baby milk powder products had very low iodine content [14]. In Europe, the United States, and Australia, the recommended dietary reference intake (DRI) of 0.15 mg/d for iodine has been established [15,16]. The DRI is set at 0.14 mg/d for adults in Taiwan [17]. For consumer health, the World Health Organization set a provisional maximum tolerable daily intake of 1.0 mg iodine/d (0.017 mg/kg body weight) from all sources [18]. The tolerable upper intake level of iodine is 1.0 mg/d for adults older than 20 years in Taiwan [17]. The upper intake level is 1.1 mg/d for adults in the United States and Australia [16]. According to Zimmermann and Andersson [19], there are 32 countries with iodine deficiency problems, more than half of which are in the industrialized world. Knowledge about iodine content in food will help consumers maintain optimal iodine consumption and protect against excessive intake. Iodine concentration in food needs to be monitored for consumer health, and this is carried out in Japan [20,21], Canada [22], Hong Kong [23], the UK [24], Australia [25], China [26], and in many other countries. Although the European Union has not issued any regulation on maximum permissible iodine levels in algae food products, France has set a limit of 5 mg/kg dry matter for iodine in edible seaweeds [1]. The Federal Institute for Risk Assessment in Germany warned that dry algae food products with more than 20 mg/kg dry weight might damage health [27]. The US Food and Drug Administration has set an upper limit of 5000 ppm for iodine in algal products on dry mass basis [1], whereas Australia has set a maximum tolerable iodine level of 1000 mg/kg dried weight in algal food [25].

Iodine is volatile and can be reduced or oxidized easily, which makes analyzing iodine content in foodstuff a challenging task. Many analytical methods have been developed for the determination of iodine content in food, including neutron activation analysis, inductively coupled plasma-optical emission spectroscopy (ICP-OES), ICP-mass spectrometry (ICP-MS) and gas chromatography-electron capture detector (GC-ECD) [28–31]. Neutron activation analysis can analyze very low iodine concentrations but is costly and requires special equipment. The sensitivity of iodine measurement by ICP-OES is not very good because of the vacuum UV emission of iodine and the interference from phosphorous emission. ICP-MS tends to have poor signal stability and memory effects for iodine detection [30]. Sample deposition on the sampler and skimmer cones and sample material buildup in the plasma torch and spray chamber could happen in ICP-MS and cause serious memory effects. Several GC-ECD methods such as pentafluoro derivatives [32] and ketone derivatives [20,21,33–35] have been developed for iodine determination. The pentafluoro derivatization reagent, however, is expensive and is not cost-effective compared to the

ketone reagent. Kikuchi et al [20] analyzed 139 kinds of food and beverages by GC-ECD via derivatization with 2-butanone. The detection limit in the study of Kikuchi et al [20] was 0.5 mg/kg. Derivatization with 2-butanone will produce two isomers. Mitsuhashi and Kaneda [33] determined iodine content in milk, wheat flour, and beef using GC-ECD via derivatization with 3-pentanone. Gu et al [34] determined iodine content in milk and oyster tissue samples by GC-ECD via derivatization with 3-pentanone. Akhoundzadeh et al [35] improved the 3-pentanone derivatization GC-ECD method with head-space single drop microextraction procedure and achieved a detection limit of 250 ng/L in infant formula.

There have been relatively few studies in relation to the iodine content of seaweeds in Taiwan, and only one study specifically reported on the iodine content of seaweeds in Taiwan [32]. The study using GC-ECD was very limited in seaweed samples. The iodine contents of three popular seaweed products in Taiwan—nori, wakame, and kombu—were thus investigated using the GC-ECD method in the present study. The seaweed samples consisted of 10 nori products, 10 wakame products, and 10 kombu products. The method presented here requires relatively low-cost GC equipment and is also suitable for other food matrices. The iodine intake from seaweed in the current survey was calculated and compared with the iodine DRI of Taiwan. The benefits of seaweed consumption are also discussed.

2. Materials and methods

2.1. Reagents and chemicals

KI (potassium iodide; puriss. p.a., reagent grade, ISO, reagent Ph. Eur., $\geq 99.5\%$), 3-pentanone (ReagentPlus, $\geq 99.0\%$), and $K_2Cr_2O_7$ (ReagentPlus, $\geq 99.5\%$) were purchased from Sigma-Aldrich (Sigma-Aldrich, St. Louis, MO, USA). NaOH (ACS, Reagent Ph. Eur.) was obtained from Merck (Darmstadt, Germany). H_2SO_4 (Extra Pure Reagent) was from Union Chemical Works Ltd. (Hsinchu, Taiwan). *n*-Hexane ($\geq 99\%$, HPLC grade) was from Tedia (Fairfield, OH, USA). Seaweed samples were purchased from local convenient stores and supermarkets. All seaweed samples were dried seaweeds. The place of origin for all seaweed samples is listed in Table 1.

2.2. Instrumentations

The Agilent 7890A gas chromatograph equipped with an ECD detector was used for the detection of derivatized product 2-iodo-3-pentanone. The HP-5MS capillary column (Agilent Technologies, Santa Clara, CA, USA) was used for chromatographic separation. The Mettler Toledo AL204 balance was used for weighing of samples. The sample ashing and carbonation were carried in the Nabertherm E4/11/R6 ashing furnace. The Corning PC620D hot plate was used for heating. Delta ultrasonic bath DC400H (Taipei, Taiwan) was used for sample dissolution. The shaker was a funnel shaker FS-12 obtained from Shin Kwang Co. (Taipei, Taiwan).

Table 1 – Iodine concentration (mg/kg) in dry seaweed products by GC-ECD.

Product type	No. 1	No. 2	No. 3	No. 4	No. 5	No. 6	No. 7	No. 8	No. 9	No. 10	Average (mg/kg)
Nori	45.8	34	40.7	29.3	33.3	39.1	37.9	36.8	31.1	41.4	36.9
Origin	Taiwan	Japan	Taiwan	Thailand	Thailand	Korea	Taiwan	Japan	Thailand	Taiwan	
Wakame	104.2	156.7	185.1	93.9	118.2	102.8	156.7	152.2	162.1	165.1	139.7
Origin	China	China	Japan	Japan	Japan	Taiwan	China	China	China	China	
Kombu	4921.3	303.2	3297.7	1764.4	2250.8	4384.8	241	2236.2	3541.6	2291.6	2523.3
Origin	Korea	Japan	Japan	Japan	Japan	Korea	Japan	China	Taiwan	Taiwan	

GC-ECD = gas chromatography-electron capture detector.

2.3. Sample pretreatment

Because iodine is volatile, the alkaline ashing technique and ketone derivatization were adopted with modifications to convert iodine into iodoketone [20,21,33]. Due to the high iodine content in seaweeds, the sample mass was kept between 0.1 and 0.2 g. After weighing 0.1–0.2 g dry seaweed sample into a porcelain crucible, 4 mL 4N NaOH solution was added. Then, 2 mL 20% KNO₃ was added to the ashing solution previously described by Mitsuhashi and Kaneda [33], but not by Kikuchi et al [20] and Kobayashi et al [21], whose procedures were followed here for preparing the ashing solution. The sample solution was heated on a hot plate into a slurry state. If the sample did not turn into a slurry state, 4 mL water was added to the crucible and the sample was heated repeatedly. The sample slurry should be kept heated until it is completely dry. The crucible with the lid on was then placed into the ashing furnace to avoid loss of iodine. A complete and smooth combustion is essential for a good recovery of iodine. The heating procedure in the present work was different from that described by Kikuchi et al [20] and Kobayashi et al [21]. Initially, the crucible was heated at 100°C for 1 hour, then at 200°C for 1 hour, and followed by sample carbonation at 500°C for 2 hours. In the previous work by Mitsuhashi and Kaneda [33] and Kobayashi et al [21], the ashing temperature was 550°C and lasted 7–8 hours, which would cause iodine loss in samples with high iodine contents such as seaweeds. The carbonation time should not exceed 2 hours to avoid iodine loss and degradation. After cooling down in a desiccator, 15 mL deionized water was added into the crucible. The sample crucible was placed on a hot plate to heat up and dissolve the ash. To aid the dissolution process, the crucible was ultrasonicated in an ultrasonic bath. The ashing solution was then suction filtered. The ash was dissolved again in 15 mL water, and the ultrasonication and suction filtering process was repeated. The filtrated solutions were then combined, and water was added volumetrically to 50 mL. A 2-mL sample solution was placed into a test tube and water was added to make up a total volume of 5 mL. The derivatization reagent—0.5 mL 3-pentanone, 1 mL 0.5% (w/v) potassium dichromate, 1 mL 50% (v/v) H₂SO₄—was added in and mixed well. The test tube was allowed to stand for 6 hours for the reaction to complete. To extract the derivatized iodoketone product, 5 mL *n*-hexane was added to the separation funnel, which was then shaken for 10 minutes. The separation funnel was allowed to stand until a clear delineation of two layers appeared. The upper hexane layer was filtered through a 0.45- μ m syringe filter and ready for GC analysis.

2.4. GC-ECD analysis

Nitrogen at a constant pressure of 11.258 psi was used as a carrier gas. The injection temperature was maintained at 260°C, and all injections were made in split mode with a split ratio of 10:1. The injection volume was 1 μ L. The GC oven temperature was held at 50°C for 3 minutes, raised to 220°C at 30°C/minute, then programmed to 250°C at 30°C/minute and held for 5 minutes. The ECD detector temperature was maintained at 300°C. The makeup gas was nitrogen at a makeup flow of 29 mL/minute. The GC chromatograms for iodine standard solution and seaweed samples are shown in Fig. 1. Seaweeds are known to contain both bromine and iodine [36], which can also react with 3-pentanone [37]. The peak at 12.08 minutes was identified as 2-iodo-3-pentanone by comparison of the GC chromatogram of the KI standard solution through the sample pretreatment step. The peak at 9.90 minutes was identified as 2-bromo-3-pentanone by comparison of the GC chromatogram using the KBr solution as a reference standard.

3. Results and discussion

3.1. Method performance validation

Method performance was validated by checking the retention time, linearity, recovery rate, within-day precision, between-day precision, and method detection limit. The sample matrix was 0.2 g nori, and the pretreatment procedure was followed to derivatize iodine with 3-pentanone for GC-ECD analysis.

3.1.1. Retention time

The retention time data for five iodine concentration levels of 0.02 μ g/mL, 0.04 μ g/mL, 0.06 μ g/mL, 0.08 μ g/mL, and 0.10 μ g/mL in triplicate for 3 days are shown in Table 2. All retention time differences are within $\pm 0.02\%$.

3.1.2. Linearity of calibration curve and limit of quantitation

The calibration curve was linear over the iodine concentration range of 0.02 μ g/mL, 0.04 μ g/mL, 0.06 μ g/mL, 0.08 μ g/mL, and 0.1 μ g/mL. The slope and the intercept of the calibration curve were 3949.62 and 66.61, respectively. The correlation coefficient of the calibration curve was 0.9958. The limit of quantitation was 0.5 mg/kg as determined by the signal-to-noise ratio at 10:1.

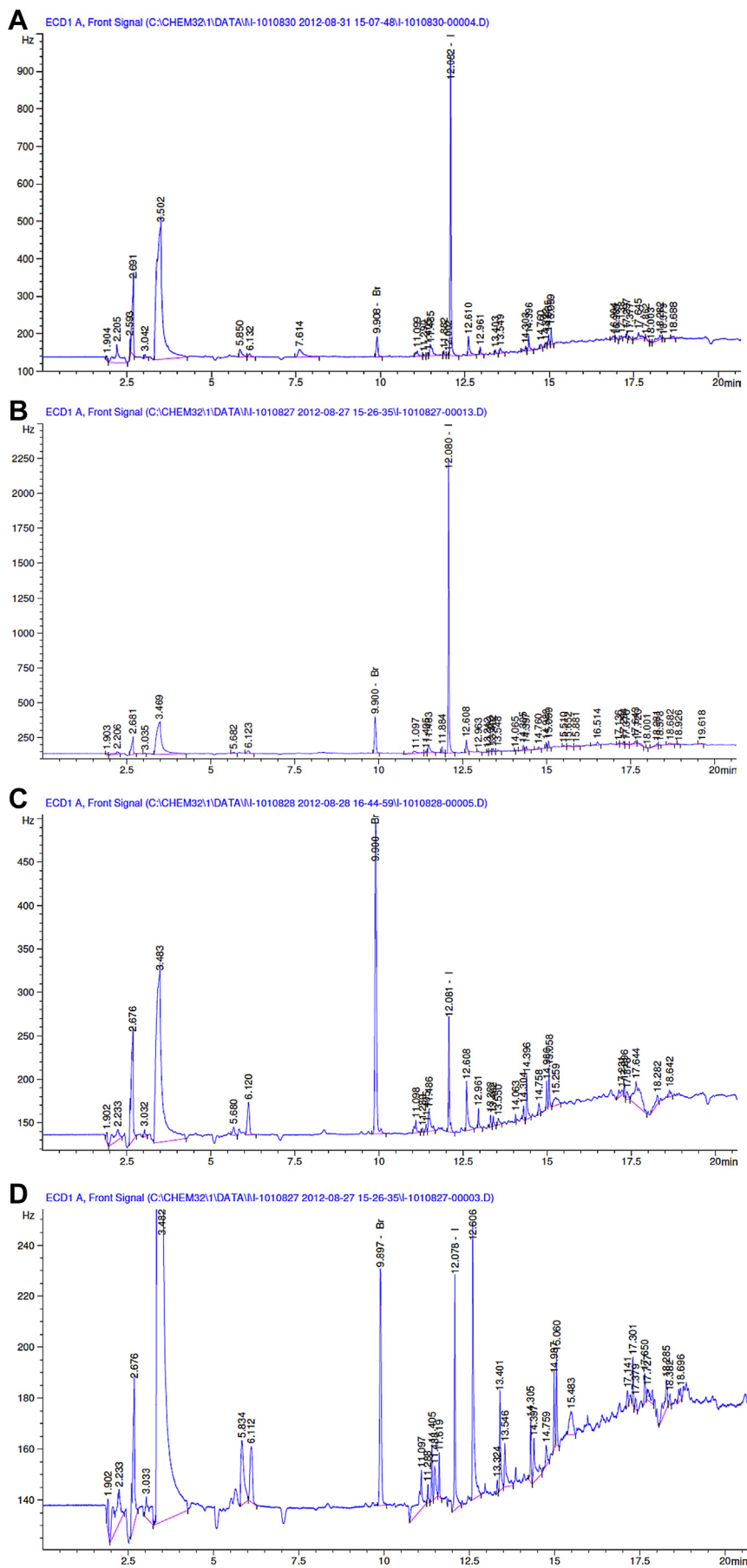


Table 2 – Retention time data for 2-iodo-3-pentanone (n = 15).

Date	Retention time ± RSD%
Day 1	12.083 ± 0.01
Day 2	12.078 ± 0.02
Day 3	12.084 ± 0.00

RSD = relative standard deviation.

Table 3 – Precision and accuracy for 2-iodo-3-pentanone (n = 3).

Concentration (µg/mL)	Recovery (%)	Within day (CV %)	Between day (CV %)
0.02	100	2.8	2.8
0.04	97.5	5.3	8.3
0.06	102	1.2	5.4
0.08	98.8	1.8	3.3
0.1	101	2.5	2.9

CV = coefficient of variation.

3.1.3. Precision and accuracy

The recovery rate and precision were examined by spiking 0.02 µg/mL, 0.04 µg/mL, 0.06 µg/mL, 0.08 µg/mL, and 0.1 µg/mL iodine standard solution into the 0.2 g nori sample in triplicate for 3 days. Table 3 shows the precision and accuracy data. The recovery rate was 97.5–101%. Both the within-day and between-day precision were less than 10%.

3.2. Iodine contents in seaweed products

The survey results for iodine content in seaweed by GC-ECD are shown in Table 1. The iodine contents surveyed for nori, wakame, and kombu were 29.3–45.8 (ave. 36.9) mg/kg, 93.9–185.1 (ave. 139.7) mg/kg, and 241–4921 (ave. 2523.3) mg/kg, respectively. Kombu has the highest average iodine content and nori the lowest. Comparison of the iodine contents found in the present study with those of other studies is shown in Table 4 [1–4,9,20]. The iodine contents in the present study are comparable to those reported by Teas et al [9]. In their work, the iodine content in nori, wakame, and kombu was 16–43 mg/kg, 66–1571 mg/kg, and 1542–5307 mg/kg, respectively, for different countries. Although the iodine content of nori in this study and that reported by Teas et al [9] was comparable, previous work by Mabeau and Fleurence [1] and Holdt and Kraan [4] have reported higher iodine contents of 20–250 mg/kg and 5–550 mg/kg, respectively. The iodine content of wakame in previous studies was comparable to the present study, except that the value reported by MacArtain et al [3] was lower and that by Teas et al [9] was higher than that in most studies. The iodine contents of kombu reported by Mabeau and Fleurence [1] and Holdt and Kraan [4] were 2000–10,000 mg/kg and 230–12,000 mg/kg, respectively. The highest iodine content in kombu reported by

Table 4 – Comparison of the iodine contents of present study with previous studies.

Study	Nori (mg/kg)	Wakame (mg/kg)	Kombu (mg/kg)
Mabeau and Fleurence [1]	20–250	250	2000–10,000
Dawczynski et al [2]	26.7–33.2	163	2934
MacArtain et al [3]	13	39	700
Holdt and Kraan [4]	5–550	60–350	230–12,000
Kikuchi et al [20]	0.7–9.4	41.1–538	25–2250
Teas et al [9]	16–43	66–1571	1542–5307
This study	29.3–45.8	93.9–185.1	241–4921

Mabeau and Fleurence [1] and Holdt and Kraan [4] was twice higher compared to the highest recorded in the present study.

3.3. Iodine intake from consumption of seaweed

To calculate the different intake scenarios for an adult, the average seaweed consumption information derived from the 1993–1996 and 2005–2008 Nutrition and Health Survey in Taiwan (NAHSIT) was adopted [38,39]. The daily seaweed consumption in NAHSIT 1993–1996 was 2.7–3.4 g according to Wu et al [40], which corresponds to 5.3–6.7 kcal of energy. The average consumption of seaweed in NAHSIT 2005–2008 expressed in energy content was 1.5 kcal and 1.9 kcal for male and female adults who are 19–64 years old, respectively. In this context, the daily seaweed consumption is 0.76 g and 0.96 g for male and female adults, respectively. The calculated iodine intakes according to NAHSIT 2005–2008 estimated seaweed consumption are listed in Table 5. As shown in Table 5, the consumption of kombu could easily exceed the RDI value of 0.14 mg/d for iodine. For male adults, the average iodine consumption value of kombu would exceed the RDI value by 1370%, whereas for women it would exceed the RDI value by 1730%. By consuming the highest iodine value of kombu, the male adult iodine intake could exceed the RDI value by 2686% and the female adult iodine intake would exceed by 3389%.

Because no seaweed consumption data were available for other age groups in Taiwan, the weight of seaweed consumption for the RDI requirement of iodine are calculated and listed in Table 6. The RDI of iodine for different age groups are adopted from the Dietary Reference Intakes Tables of Taiwan [17]. As shown in Table 6, the consumption of 1.76–3.79 g nori based on their average iodine concentration would satisfy the daily requirement for iodine for different age groups. By contrast, for kombu, based on their average iodine concentration, a person only needs to consume 0.03–0.06 g to meet the RDI requirement. Based on the calculation, nori could be a good source for iodine supplement. This also reflects the fact that the nori sheet is popular as snack food in Asia.

3.4. Benefits of seaweed consumption

Salt iodization is the method used by most countries to combat iodine deficiency disorders [19]. However, salt has

Figure 1 – Gas chromatogram for iodine standard solution and seaweed samples. (A) 0.1 µg/mL iodine standard solution, (B) kombu, (C) wakame, and (D) nori.

Table 5 – Dietary iodine intake (mg) from consumption of dry seaweed in this study and % RDI [17] value of Taiwan.

	Average seaweed consumption (g/d)	36.9 ppm ^a (% RDI)	139.7 ppm ^b (% RDI)	2523.3 ppm ^c (% RDI)	45.8 ppm ^d (% RDI)	185.1 ppm ^e (% RDI)	4921.3 ppm ^f (% RDI)
Male	0.76	0.028 (20)	0.106 (76)	1.918 (1370)	0.035 (25)	0.141 (101)	3.761 (2686)
Female	0.96	0.035 (25)	0.134 (96)	2.422 (1730)	0.044 (32)	0.178 (127)	4.745 (3389)

RDI = recommended daily intake.

^a Average iodine content in nori.

^b Average iodine content in wakame.

^c Average iodine content in kombu.

^d Highest iodine content in nori.

^e Highest iodine content in wakame.

^f Highest iodine content in kombu.

long been known to elevate blood pressure, which is the most important cause for 62% of strokes and 49% of coronary heart diseases [41]. According to NAHSIT 2005–2008 [39], salt intake was 11.4 g in men and 8.0 g in women aged 19–64 years, which is much higher than the salt DRI of 6.0 g, and salt intake should be reduced. Reducing salt intake has become a global trend to decrease the risk of cardiovascular diseases. Seaweed with its high iodine content could be useful to remedy dietary iodine deficiency without the side effect of hypertension brought on by salt. Seaweed consumption has been shown to lower blood pressure as revealed in the studies of Bocanegra et al [6], Fitzgerald et al [7], and Wada et al [42]. Bocanegra et al [6] linked the hypotensive effects to the dietary fiber found in seaweeds, and Fitzgerald et al [7] ascribed the antihypertensive effects to the bioactive peptides in seaweeds. Sobko et al [43] attributed the blood pressure-lowering effect of seaweeds to their rich nitrate content [43] and noted that the seaweed laver could contain nitrates as high as 3990 ± 3940 mg/kg.

Seaweeds can be contaminated with heavy metals because of bioaccumulation. The heavy metal contents in most edible seaweeds are generally below the maximum concentrations permitted in most countries [1,3,6]. Heavy metals could be a safety risk for seaweed consumption, but most countries regularly monitor the heavy metal contents in seaweed and seafood products. In this respect, the food safety risk of seaweeds in relation to dangerously high heavy metal levels is low.

According to the most recent iodine nutrition status survey in Taiwan, the median urinary iodine level for adults older than 19 years was 99.6 µg/L [44]. The median urinary iodine

level was 102.5 µg/L and 97.7 µg/L for male and female adults, respectively. The median urinary iodine level for senior adults older than 65 years was 99.3 µg/L and 77.4 µg/L for men and women, respectively. The survey also showed that both female adults and senior female adults in Taiwan did not have sufficient dietary intake of iodine, and that this deficiency should be resolved via iodine supplements through food consumption. Seaweed can be used as nutrition supplements for female adults with iodine deficiency. Iodine fortification with seaweeds of high iodine content will not cause hyperthyroidism if the seaweed is prepared by boiling in soup with abundant goitrogenic vegetables.

In conclusion, in this study, a cost-effective method for detecting iodine content in seaweed using GC-ECD has been developed. The iodine content surveyed was 29.3–45.8 mg/kg for nori, 93.9–185.1 mg/kg for wakame, and 241–4921 mg/kg for kombu. Kombu had the highest average iodine content (2523.5 mg/kg), followed by wakame (139.7 mg/kg) and nori (36.9 mg/kg).

Consumption of kombu could easily exceed the DRI value for iodine (0.14 mg/d). Algal products with different iodine concentrations need to be taken into account in the nutritional survey for health hazards and benefits evaluation. To prevent excessive consumption, it is imperative for consumers to be knowledgeable about the iodine contents in different food groups. Adequate consumption of seaweed is beneficial for health. Iodine fortification with seaweeds of high iodine content will not cause hyperthyroidism if the seaweed is prepared by boiling in soup with abundant

Table 6 – Weight of dry seaweed (g) consumption for the RDI [17] requirement of iodine.

Age (y)	RDI (µg/d)	36.9 ppm ^a	139.7 ppm ^b	2523.3 ppm ^c	45.8 ppm ^d	185.1 ppm ^e	4921.3 ppm ^f
1–3	65	1.76	0.47	0.03	1.42	0.35	0.01
4–6	90	2.44	0.64	0.04	1.97	0.49	0.02
7–9	100	2.71	0.72	0.04	2.18	0.54	0.02
10–12	110	2.98	0.79	0.04	2.40	0.59	0.02
13–15	120	3.25	0.86	0.05	2.62	0.65	0.02
16–18	130	3.52	0.93	0.05	2.84	0.70	0.03
19–70	140	3.79	1.00	0.06	3.06	0.76	0.03

RDI = recommended daily intake.

^a Average iodine content in nori.

^b Average iodine content in wakame.

^c Average iodine content in kombu.

^d Highest iodine content in nori.

^e Highest iodine content in wakame.

^f Highest iodine content in kombu.

goitrogenic vegetables. Further studies should be conducted to understand the bioavailability of iodine in humans and to comprehensively profile the goitrogenic contents in different food groups.

Conflicts of interest

None.

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