High Iron Content and Bioavailability in Humans from Four Species of Marine Algae[1,2]

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Abstract

Searching for economical, nonconventional sources of iron is important in underdeveloped countries to combat iron deficiency and anemia. Our objective was to study iron, vitamin C, and phytic acid composition and also iron bioavailability from 4 species of marine algae included in a rice-based meal. Marine algae (*Ulva sp*, *Sargassum sp*, *Porphyra sp*, and *Gracilariopsis sp*) were analyzed for monthly variations in iron and for ascorbic acid and phytic acid concentrations. A total of 96 subjects received rice-based meals containing the 4 species of marine algae in different proportions, raw or cooked. All meals contained radioactive iron. Absorption was evaluated by calculating the radioactive iron incorporation in subjects' blood. Iron concentrations in algae were high and varied widely, depending on the species and time of year. The highest iron concentrations were found in *Sargassum* (157 mg/100 g) and *Gracilariopsis* (196 mg/100 g). Phytates were not detected in the algae and ascorbic acid concentration fluctuated between 38 μg/g dry weight (*Ulva*) and 362 μg/g dry weight (*Sargassum*). Algae significantly increased iron absorption in rice-based meals. Cooking did not affect iron absorption compared with raw algae. Results indicate that *Ulva sp*, *Sargassum sp*, *Porphyra sp*, and *Gracilariopsis sp* are good sources of ascorbic acid and bioavailable iron. The percentage of iron absorption was similar among all algae tested, although *Sargassum sp* resulted in the highest iron intake. Based on these results, and on the high reproduction rates of algae during certain seasons, promoting algae consumption in some countries could help to improve iron nutrition.   J. Nutr. 137: 2691–2695, 2007.

Introduction

The consumption of marine algae has been very important nutritionally for many cultures that developed near the sea or that depend on marine products for food (1–3). In their natural state, algae contain 80–90% water. On a dry weight basis, ~50% are carbohydrates, 1–3% lipids, and 7–38% minerals. Protein content is highly variable (10–47%), with a high proportion of essential amino acids (4,5). Algae's high concentration of vitamins and minerals represents an obvious health benefit. Algae contain more vitamin A, B-12, and C, β-carotene, pantothenate, folate, riboflavin, and niacin than fruits and vegetables from regular land cultivars (6,7).

Iron deficiency is a nutritional problem that affects many people all over the world. It primarily occurs as a result of insufficient intake of bioavailable iron; infections; deficiencies of vitamin A, folic acid, or vitamin B-12; and increased requirements during growing periods and pregnancy or augmented losses in women during reproductive age (8). Some reports indicate that marine algae are good sources of iron, although this does not necessarily mean bioavailable iron. A study of 59 marine algae showed iron concentrations ranging from 52 to 3410 mg/kg. Based on these values, the daily iron requirement would be met with small amounts of algae in the diet (9).

Many tropical countries posses a rich, varied, and exuberant marine flora that has been poorly exploited and studied as an alternative source of nutrients, such as iron. However, because a high iron content does not necessarily imply a high iron utilization, our objective was to study the nutrient composition, specifically iron, vitamin C, and phytic acid contents, of 4 species of marine algae collected in Venezuelan coasts and consumed worldwide and also to evaluate iron bioavailability from these 4 algae when included in a rice-based meal.

Materials and Methods

Selection of algae for human consumption. Algae were collected from 4 beaches in Margarita Island, Venezuela by swimming and diving by trained personnel from Biotecmar CA. Material collected was classified as green, red, and brown algae and washed with seawater, eliminating impurities such as sand, rock, epiphytes, and epifauna. Algae were sun dried for 6 h and for a further 12 h in the shade before they were packed, labeled, and refrigerated at 4°C until being transported to the laboratory, where samples were freeze-dried to constant weight after eliminating any sand or rock residue.

Seasonal variation of iron concentrations in the algae selected for human consumption. Four algae were selected for iron bioavailability studies with rice. These species (*Ulva sp*, *Sargassum sp*, *Porphyra sp*, and

Gracilariopsis sp) were analyzed for monthly variations in iron content for 2 y. With the exception of *Porphyra sp*, which grows in the area studied only between December and April, samples were taken monthly (except for May and December) for the other algae analyzed. Samples were collected as described previously and once in the laboratory were washed by immersion in tap water 5 times, followed by soaking for 30 min with a water change at 15 min. To eliminate excess water, samples were placed on paper towels for 1 h. At this stage, samples were taken for total iron determinations and results reported as mg iron/100 g wet weight or hydrated algae. The remaining samples were frozen and freeze-dried to constant weight and results expressed as mg iron/100 g dried weight algae. Total iron was determined by the acid digestion method with colorimetric detection (10).

Ascorbic acid measurement. Ascorbic acid content in algae was measured by the Ferrozine method (11), which is based on the reduction of iron by ascorbate. Two moles of ferrous iron are produced per mole of ascorbic acid, which are measured by color development with 3-(2-piridyl 5,6-bis(phenylsulfonic acid)-1,2,4-triazine) (Ferrozine) at 562 nm.

Phytic acid measurement. Phytic acid content in algae was measured by the method of Hang and Lantzsch (12). Briefly, extracted samples reacted with an iron solution of known concentration and, after precipitation of phytate-iron complexes, the iron remaining in the supernatant was measured and compared with a standard curve to indirectly measure phytate content.

Meal preparation. Six iron absorption studies were performed using 3 or 4 species of algae. In each absorption study, each subject received 4 meals. The first 4 studies were performed with *Ulva sp*, *Sargassum sp*, *Porphyra sp*, and *Gracilariopsis sp* and studies 5 and 6 contained only *Ulva sp*, *Sargassum sp*, and *Porphyra sp*.

All studies included a 180-g portion of cooked rice with 5 g of margarine and a glass of water. Radioactive tagging (^{59}Fe or ^{55}Fe) was added to the water used to cook the rice. The algae were added to the different meals according to the protocol varying in species, proportions, and cooking state.

In studies 1–4, iron absorption was evaluated in the presence of 4 species of algae administered in 2 doses (10 or 20 g). We examined the effect of cooking the algae on iron absorption with 20-g doses in 2 different meals. In one of the meals, algae was cooked with the rice and in another meal, 20 g raw algae was added, in small pieces, to the cooked rice. The absorption of iron from 10-g doses was evaluated only with raw algae, which were added to the cooked rice portions. Study 1 included the following: Meal 1, radioactive rice meal alone; Meal 2, radioactive rice meal with 20 g of *Gracilariopsis sp* cooked with the rice; Meal 3, radioactive rice meal with 10 g of *Gracilariopsis* added raw to the cooked rice; and Meal 4, radioactive rice meal with 20 g of *Gracilariopsis sp* added raw to the cooked rice. Studies 2, 3, and 4 were similar except for the algae tested, which included *Ulva sp* for study 2, *Sargassum sp* for study 3, and *Porphyra sp* for study 4.

In Studies 5 and 6, algae species (*Porphyra sp*, *Ulva sp*, and *Sargassum sp*) were administered to the same subject on different occasions. Each meal contained 15 g algae. In study 5, algae were administered raw to the cooked rice and in study 6, algae were cooked with the rice: Meal 1, Radioactive rice meal alone; Meal 2, radioactive rice meal with 15 g *Porphyra sp* (raw for study 5 and cooked for study 6); Meal 3, radioactive rice meal with 15 g *Ulva sp* (raw or cooked); and Meal 4, radioactive rice meal with 15 g *Sargassum sp* (raw or cooked).

Bioavailability studies. Ninety-six human subjects (23 men and 73 women) aged 14–50 y voluntarily participated in this study. The subjects belonged to the low socioeconomic stratum and were in apparent good health, but some had moderate iron deficiency anemia, based on WHO cutoff points (8). The Ethics Committee for the Protection of Human Subjects of the Venezuelan Institute for Scientific Research approved the studies and each volunteer signed a written consent form.

We performed 6 absorption studies consisting of 4 meals per study. Each subject was allowed to participate in only 1 study. Each study included ~20 volunteers. The first day of the experiment, pregnancy tests were performed and all selected individuals were informed about the objectives and procedures of the study.

The protocol for all studies was as follows: Meal 1, tagged with ^{59}Fe (0.9 μCi per person), was administered to the subjects after an overnight fast. Meal 2, also extrinsically labeled but with ^{55}Fe (1.3 μCi per person), was consumed 4 h later. No food or drink (except for water) was allowed between Meals 1 and 2 and for 4 h after administration of Meal 2. The protocol for the administration of radioactive food in the morning after an overnight fast and the afternoon of the same day was based on experiments previously published (13).

On d 15, blood (30 mL) was drawn to determine the hematological profile [hemoglobin concentration (14), serum iron (15), unsaturated binding capacity (16), and serum ferritin concentration (17)] and to measure radioactivity incorporation into red cells. Duplicate blood (10 mL) and triplicate samples of radioactive food were prepared for radioactive counting using the technique of Dern and Hart (18,19). Iron absorption from each meal was calculated from the radioactivity in the subject's blood, using an estimation of blood volume based on sex, weight, and height (20).

On the same day (d 15) Meals 3 and 4 were administered following the same protocol for Meals 1 and 2. On d 30, we collected a blood sample to measure radioactivity incorporation and serum ferritin concentration.

Statistical analysis. Data analysis was based on comparisons (repeated measures ANOVA with Bonferroni as a post-test) of absorption values for the 4 meals in each study, comparing doses of algae and cooking procedures for paired data. We performed *t* tests for nonpaired data for comparisons among algae species. Means and SD were calculated for all anthropometrical and hematological measurements. Geometric means and SE were calculated for all absorption data and ferritin concentrations. The statistical analysis was performed using GraphPad Instat and Excel programs. Values in the text are means ± SD.

Results

Changes in weight of hydrated or dry samples. Dry algae collected from different beaches were washed and hydrated for 30 min. The weight registered at that stage was considered as wet or fresh weight. All chemical determinations were performed on a dry basis after freeze-drying algae samples. Ratios for weight lost by liophylization were calculated (wet weight:dry weight) in at least 20 replicates for each algae, and the factors for each species were as follows: *Sargassum*, 2.1; *Ulva*, 2.3; *Porphyra*, 3.1; and *Gracilariopsis*, 3.9, indicating a water content of 53, 57, 68, and 74%, respectively. We used these factors to calculate the iron content of the meals administered and also to determine the amount of iron absorbed by each subject participating in iron absorption studies.

Iron composition of selected algae. Considering the results from an initial screening for iron content, algae abundance, and difficulties imposed by geographic limitations, *Sargassum sp*, *Ulva sp*, *Porphyra sp*, and *Gracilariopsis sp* were selected to study month-by-month variations in iron content. Iron concentration varied considerably among algae species and also among the months evaluated (**Table 1**). Iron concentrations in the 4 species of algae were as follows: *Sargassum*, 156.9 ± 32.4 mg iron/100 g dry weight; *Ulva*, 57.5 ± 31.1 mg iron/100 g dry weight; *Porphyra*, 15.5 ± 2.9 mg iron/100 g dry weight; and *Gracilariopsis*, 195.9 ± 54.9 mg iron/100 g dry weight. The months in which algae had highest iron concentration were July for *Sargassum sp* and *Gracilariopsis sp*, August for *Ulva sp*, and April for *Porphyra sp*.

Ascorbic acid concentrations in algae. Ascorbic acid concentrations were 37.8 μg/g dry weight for *Ulva sp*, 232.28 ± 48.84

TABLE 1 Seasonal variation of iron concentration in algae (*Sargassum sp*, *Ulva sp*, *Porphyra sp*, and *Gracilariopsis sp*) selected for human consumption and collected on the coast of Margarita Island, Venezuela[1]

Algae	January	February	March	April	June	July	August	September	October	November	December
					mg iron/100 g dry algae						
Sargassum sp	81.33 ± 18.83	ND[2]	ND	135.33 ± 57.05	ND	289.50 ± 70.72	240.33 ± 9.87	160.50 ± 35.03	97.67 ± 16.26	93.67 ± 19.35	ND
Ulva sp	32.40 ± 10.05	62.08 ± 21.65	48.77 ± 29.81	51.64 ± 32.37	21.00 ± 1.00	47.40 ± 46.07	236.60 ± 147.27	21.67 ± 3.06	28.23 ± 16.01	25.00 ± 3.46	ND
Porphyra sp	14.05 ± 2.16	11.57 ± 0.14									
	15.67 ± 2.08	12.37 ± 2.87	17.78 ± 4.81	38.67 ± 5.03	ND	ND	ND	ND	ND	ND	17.80 ± 2.29
	13.87 ± 4.24										
Gracilariopsis sp	170.82 ± 42.00	293.89 ± 113.53	97.17 ± 50.14	97.29 ± 83.57	ND	360.67 ± 55.58	123.33 ± 8.50	213.70 ± 75.40	279.67 ± 26.63	126.50 ± 38.48	ND

[1] Values are means ± SD of samples collected at a minimum of 4 beaches, $n = 3–18$. For *Porphyra sp*, sampling was performed during 3 consecutive years ($n = 3–18$).
[2] ND, Not determined.

μg/g dry weight for *Porphyra*, 250.65 ± 226.87 μg/g dry weight for *Gracilariopsis*, and 362.44 ± 41.83 μg/g dry weight for *Sargassum*. The number of experiments performed in each algae species was 18, 20, 8, and 20, respectively. The fluctuation in ascorbic acid concentration was less than that of the iron concentration despite the known susceptibility of this vitamin to storage, processing, and exposure to light or air.

Phytic acid content. Phytic acid was undetectable in all the algae tested by the method used in this study, with a lower limit of detection of 30 mg/L.

Meal preparation. Despite their initial surprise at the combination of rice with raw or cooked algae, volunteers accepted and completely consumed the meal and returned on 2 other occasions to finish the study. Rice with added *Gracilariopsis sp* was not as well accepted, because the algae looked like little sticks and was hard to eat. Due to its high iron and ascorbic acid concentrations, *Gracilariopsis sp* was an excellent candidate for absorption studies. However, because it was not accepted by volunteers in bioavailability studies, it was eliminated from subsequent tests. The iron content of rice meals administered containing 10, 15, or 20 g algae was 3.1, 4.4, and 5.6 mg for *Ulva sp*; 8.1, 11.8, and 15.6 mg for *Sargassum sp*; and 1.1, 1.4, and 1.6 mg for *Porphyra sp*.

All groups were similar in terms of predominance of females over males, as well as the other anthropometrical and hematological characteristics (**Table 2**).

Hemoglobin concentrations were comparable among groups and that there were no anemic or iron-deficient groups based on hemoglobin and ferritin concentrations, respectively. However, when analyzed subject by subject, we found that 2, 3, 4, 2, 5, and 6 individuals were anemic in groups 1–6, respectively, whereas 4, 4, 2, 5, 4, and 6 presented iron deficiency.

Bioavailability studies. Including any of the 4 species of algae selected significantly increased iron absorption compared with the rice meal alone (**Table 3**). Iron absorption from meals containing 10 or 20 g of algae was not different, except for *Porphyra sp*, with a significantly lower absorption with 10 g compared with 20 g. The effect of the cooking procedure on iron absorption was also evaluated in these experiments. The effect of including 20 g of algae in the water for cooking rice did not differ from the effect of adding 20 g of raw algae to the cooked rice, although iron absorption tended to be higher when subjects consumed cooked algae in all cases analyzed ($P = 0.063$ to 0.097). For the rice meal containing 20 g of cooked algae, the highest percentage of absorption was obtained with *Sargassum sp* (22%) and the lowest with *Gracilariopsis sp* (12%).

In the other bioavailability studies performed (*Ulva sp*, *Sargassum sp*, or *Porphyra sp*), each individual received 15 g of the 3 algae on different occasions (**Table 4**). Iron absorption increased significantly with the addition of algae compared with the rice meal alone, although absorption did not differ among the 3 algae administered. As reported for the previous 4 studies, the cooking procedure did not affect iron bioavailability, but values

TABLE 2 Anthropometric and baseline hematological characteristics of subjects participating in iron absorption studies from preparations of algae and rice[1]

	Study 1	Study 2	Study 3	Study 4	Study 5	Study 6
n	11	18	15	14	20	18
Sex	9 F, 2 M	12 F, 6 M	10 F, 5 M	9 F, 5 M	16 F, 4 M	17 F, 1 M
Age, *y*	32.8 ± 14.8	39.4 ± 15.2	35.3 ± 18.6	36.3 ± 16.3	35.3 ± 14.5	28.0 ± 15.1
Weight, *kg*	55.2 ± 17.3	65.9 ± 12.3	59.4 ± 6.3	60.7 ± 11.8	63.5 ± 12.7	61.1 ± 9.9
Height, *cm*	159 ± 0.07	160 ± 0.09	160 ± 0.06	160 ± 0.08	163 ± 0.06	164 ± 0.08
Hemoglobin, *g/L*	133 ± 11	133 ± 14	133 ± 13	133 ± 13	129 ± 11	125 ± 10
Hematocrit, *L/L*	0.39 ± 0.02	0.40 ± 0.04	0.40 ± 0.04	0.40 ± 0.03	0.40 ± 0.04	0.36 ± 0.02
Serum iron, *μmol/L*	18.4 ± 4.5	16.4 ± 5.2	14.1 ± 4.9	16.6 ± 4.7	13.3 ± 4.1	18.0 ± 5.4
UIBC[2], *g/L*	2.23 ± 0.56	2.17 ± 0.55	2.46 ± 0.51	2.32 ± 0.57	2.78 ± 0.84	2.30 ± 0.57
TIBC[2], *g/L*	3.24 ± 0.36	3.09 ± 0.39	3.24 ± 0.41	3.24 ± 0.48	3.52 ± 0.69	3.22 ± 0.39
Transferrin saturation, %	32 ± 9	30 ± 10	24 ± 9	29 ± 11	22 ± 9	29 ± 11
Ferritin, *μg/L*	17 ± 3	22 ± 3	20 ± 2	23 ± 4	20 ± 4	18 ± 3

[1] Values are means ± SD.
[2] UIBC, Unsaturated iron-binding capacity; TIBC, total iron-binding capacity.

TABLE 3 Effect of cooking and different concentration of algae (*Gracilariopsis sp, Ulva sp, Sargassum sp,* and *Porphyra sp*) on iron absorption from a rice based meal in humans[1]

Study	Algae	*n*	Serum ferritin	Rice meal	Rice meal + 20 g cooked algae	Rice meal + 10 g raw algae	Rice meal + 20 g raw algae
			μg/L	\u2014\u2014\u2014\u2014\u2014\u2014\u2014\u2014\u2014\u2014\u2014 *% iron absorption* \u2014\u2014\u2014\u2014\u2014\u2014\u2014\u2014\u2014\u2014\u2014			
1	*Gracilariopsis*	11	17 ± 3	4.7 ± 1.7[a]	12.2 ± 1.8[b]	12.9 ± 1.7[b]	10.2 ± 2.9[b]
2	*Ulva*	18	22 ± 3	6.5 ± 1.9[a]	18.7 ± 1.3[b]	19.2 ± 1.5[b]	12.3 ± 1.8[b]
3	*Sargassum*	15	20 ± 2	4.7 ± 1.8[a]	22.0 ± 1.4[b]	13.8 ± 1.7[b]	18.8 ± 1.4[b]
4	*Porphyra*	14	23 ± 4	3.4 ± 1.9[a]	17.3 ± 2.4[b]	6.4 ± 2.0[c]	14.9 ± 1.8[b]

[1] Values are means ± SD. Iron absorption means in a row without a common letter differ, $P < 0.05$.

tended to be higher when subjects consumed cooked algae compared with raw samples ($P = 0.063$ to 0.097).

Percentages of iron absorption did not differ when subjects consumed the various samples of algae. When calculated as doses of iron absorbed from algae, based on the mean iron content of each meal and their percentage of absorption, the amount of iron absorbed was 0.17 mg from *Porphyra sp*, 0.50 mg from *Ulva sp*, and 1.75 mg from *Sargassum sp*. Results were similar for subjects who consumed cooked algae, with 0.28 mg iron from *Porphyra sp*, 0.56 mg iron from *Ulva sp*, and 2.33 mg iron from *Sargassum sp*.

Discussion

The variety, abundance, and size of algae species found in the Caribbean is noteworthy compared with other regions. The tropical zone is ideal for the development of marine farms that represent an alternative opportunity for community economic growth, generating not only food but also jobs for their habitants (21–24). The algae evaluated in this study had highly variable iron concentrations, but could be considered as good sources of iron. In general, it has been reported that the peaks of highest iron concentration matched the moments of maximum growth for each species of algae (24). In this work, the wide variation in iron content during the year and the increase during certain months seemed related to the reproductive cycle of algae and also to periods of higher water temperatures.

From the algae selected for absorption studies, *Gracilariopsis sp* and *Sargassum sp* had the highest iron content, followed by *Ulva sp* and *Porphyra sp*. Despite the high iron content found in this and previous reports for some species of algae (24,25), iron bioavailability has not been studied before, to our knowledge. In this work, we demonstrated that the inclusion of marine algae in a widely consumed food such as rice is not only feasible but also desirable to increase iron intake. The 4 species of algae initially evaluated, and the 3 finally selected, were excellent sources of iron. Although the chemical form of the iron present in algae and

its incorporation to the nonheme iron pool has not been assessed, the bioavailability of this iron seemed to be high, and increased apparent iron absorption up to 5-fold of the absorption value of the rice meal alone. This was probably due to the high vitamin C concentration and also to the low or nonexistent phytate content.

The cooking procedures did not significantly affect iron bioavailability, although absorption values were consistently higher for cooked than for raw algae. The administration of 3 different algae to the same individual allowed us to control the absorption fluctuations due to individual variations, and those experiments revealed a similar percentage of absorption for all algae tested. However, when the iron absorption was calculated according to the iron content of each algae, *Sargassum sp* was the best source of iron, with a 15-g dose meeting >100% of the daily iron requirement of 1–2 mg. *Ulva sp* supplied ~0.7 mg of iron and *Porphyra sp* contributed ~0.3 mg of iron.

It is important to highlight the high content of ascorbic acid in the algae, despite the manipulation and processing of the algae for the experiments, which suggests that these species are good, nonconventional sources of vitamin C. When analyzed on a wet weight basis, ascorbic acid concentrations of algae were comparable to some nonmarine vegetables that are considered good sources of vitamin C, such as tomatoes, spinach, peas, and some tubers, with 25, 34, 25, and 18 mg/100 g fresh food, respectively (26). It is possible to satisfy 100% of the daily dietary recommendation for vitamin C by consuming 43 g of *Ulva sp*, 7 g of *Porphyra sp*, or only 3 g of *Sargassum sp*. The role of ascorbic acid needs further investigation to evaluate the minimum concentration required to improve iron absorption from algae. Also, the low or nondetectable content of phytic acid in the species studied suggest an additional benefit of algae consumption in terms of iron availability.

It is important to evaluate other algae species, especially those growing uncontrollably in many tropical coasts. The use of these algae, with appropriate nutritional education, could help solve ecological and iron deficiency problems in some underdeveloped countries.

TABLE 4 Iron absorption in humans from rice based diets containing different species of raw or cook marine algae (*Porphyra sp, Ulva sp,* and *Sargassum sp*)[1]

Study	Algae	*n*	Serum ferritin	Rice meal	Rice meal + 15 g *Porphyra*	Rice meal + 15 g *Ulva*	Rice meal + 15 g *Sargassum*
			μg/L	\u2014\u2014\u2014\u2014\u2014\u2014\u2014\u2014\u2014\u2014\u2014 *% iron absorption* \u2014\u2014\u2014\u2014\u2014\u2014\u2014\u2014\u2014\u2014\u2014			
5	Raw	20	20 ± 4	4.9 ± 1.9[a]	12.2 ± 1.3[b]	11.3 ± 1.6[b]	14.8 ± 1.8[b]
6	Cooked	18	18 ± 3	5.3 ± 1.6[a]	19.8 ± 1.3[b]	13.9 ± 1.5[b]	19.8 ± 1.7[b]

[1] Values are means ± SD. Iron absorption means in a row without a common letter differ, $P < 0.05$.

Literature Cited

1. Xia B, Abbott I. Edible seaweeds of China and their place in the Chinese diet. Econ Bot. 1987;41:341–53.
2. Noda H. Health benefits and nutritional properties of nori. J Appl Phycol. 1993;5:225–58.
3. Nisizawa K, Noda R, Kikuchi R, Watanabe T. The main seaweed foods in Japan. Proc Int Seaweed Symp. 1987;12:5–29.
4. Kagawa A. Standard tables of food composition in Japan. In: Amino-acids composition in Japanese foods. 4th ed. Res Council Science and Tech Agency, Editors. Mie (Japan): 1983. p. 253–9.
5. Rúperez P. Mineral content of edible marine seaweeds. Food Chem. 2002;79:23–6.
6. Kanazawa I. Vitamins in algae. Bull Jpn Soc Sci Fish. 1963;29:713–31.
7. Ishii T, Susuki H, Koyanagi T. Determination of trace elements in marine organisms. 1. Factors for variation of concentration of trace element. Bull Jpn Soc Sci Fish. 1978;44:155–62.
8. FAO/WHO Expert group. Requirements of vitamin A, iron, folate and vitamin B-12. FAO Food and Nutrition Series. No. 23. FAO, Rome; 1988
9. McConnaughey E. Sea vegetables: harvesting guide and cookbook. Happy Camp (CA): Naturegraph Publishers; 1985. p. 17–36.
10. Bothwell T, Carlton R, Cook J, Finch C. Iron metabolism in man. Oxford: Blackwell Scientific Publications; 1979.
11. McGown E, Rusnak M, Lewis C, Tillotson J. Tissue ascorbic acid analysis using ferrozine compared with the dinitrophenylhydrazine method. Anal Biochem. 1982;119:55–61.
12. Haug W, Lantzch H. Sensitive method for the rapid determination of phytate in cereal products. J Sci Food Agric. 1983;34:1423–6.
13. Taylor PG, Martínez-Torres C, Méndez-Castellano H, Jaffé W, López M, Landaeta M, Leets I, Tropper E, Ramírez J, et al. Iron bioavailability from diets consumed by different socioeconomic strata of the Venezuelan population. J Nutr. 1995;125:1860–8.
14. Crosby W, Munn J, Furth F. Standardizing a method for clinical hemoglobinometry. US Armed Forces Med J. 1954;5:693–703.
15. International Committee for Standardization in Hematology (ICSH). Recommendation for measurement of serum iron in human blood. Br J Haematol. 1978;38:291–4.
16. International Committee for Standardization in Hematology (ICSH). The measurement of total and saturated iron-binding capacity in serum. Br J Haematol. 1978;38:281–90.
17. Flowers CA, Kuizon M, Beard S, Skikne B, Covell A, Cook J. A serum ferritin assay for prevalence studies of iron deficiency. Am J Hematol. 1986;23:141–51.
18. Dern RJ, Hart W. Studies with doubly labeled iron. I. Simultaneous liquid scintillation counting isotopes of ^{55}Fe and ^{59}Fe as ferrous perchlorate. J Lab Clin Med. 1961;57:322–30.
19. Dern RJ, Hart W. Studies with doubly labeled iron. II. Separation of iron from blood samples and preparation of ferrous perchlorate for liquid scintillation counting. J Lab Clin Med. 1961;57:460–7.
20. Nadler S, Hidalgo J, Bloch T. The Tulane table of blood volume in normal men. Surgery. 1962;51:224–32.
21. Racca E, Hurtado R, Dawes C, Balladares C, Rubio J. Desarrollo de cultivo de Gracilarias en la Península de Araya, Venezuela. (Development of *Gracilaria* cultivars in the Araya Peninsula). Documento de Campo N° 13. Proyecto Aquila II, GCP/RLA/102/ITA. Universidad de Oriente. Cumaná, Edo. Sucre (Venezuela):1993. p. 39–46.
22. Rincones R. Experimental cultivation of an agarophyte, *Gracilaria cornea* in the nor-east of Venezuela. In: de Oliveira EC, Kautsky N, editors. University of Sao Paulo: International Foundation for Science; 1990. p. 65–7.
23. Rincones R, Rubio J, Racca E. *Gracilaria* pilot farming in Venezuela. In: Mishigeni KD, ed. Proceedings of International Workshop on Sustainable Seaweed Research Development. Windhoek (Namibia): KD Mishigeni; 1993. p. 309–18.
24. Chapman V, Chapman D. Seaweeds and their uses. 3rd ed. New York: Chapman and Hall; 1980. p. 260
25. Norziah M, Ching C. Nutritional composition of edible seaweed *Gracilaria changgi*. Food Chem. 2000;68:69–76.
26. Instituto Nacional de Nutrición (INN). Tabla de composición de alimentos para uso practico. Publicación no. 54. Cuadernos azules. Caracas (Venezuela): Editorial Texto; 1999.