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Original Research Article

Mineral nutrient content and iron bioavailability in common and Hawaiian seaweeds assessed by an *in vitro* digestion/Caco-2 cell model

Shireen R.L. Flores [1], Joannie Dobbs, Michael A. Dunn *

Department of Human Nutrition, Food and Animal Sciences, University of Hawaii, 1955 East West Road, Honolulu, HI 96822, USA

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ABSTRACT

Iron (Fe) deficiency remains a public health problem. Deficiency is often due to low iron intake and/or iron bioavailability. Seaweeds are known to be rich in iron, but its bioavailability is poorly understood. Our objective was to evaluate common, edible seaweeds from Asia, Hawaii, and Maine as sources of iron by comparing iron content and bioavailability to spinach. Ten of 13 seaweeds analyzed contained more iron than spinach. Iron content ranged from 73 to 3490 μg/g dry matter (DM). Twenty percent of the Daily Value for iron could be obtained from just 4.3 g of dry sea lettuce, 5.1 g of dry rockweed, 9 g of dry wakame, or 13 g of dry nori. However, *in vitro* bioavailability studies indicated that, due to low absorption efficiencies, not all seaweeds would provide greater amounts of bioavailable Fe than spinach. Notable exceptions were nori and sea lettuce which provided 3 and 5-fold more bioavailable Fe, respectively. In addition, bioavailability was significantly enhanced by vitamin C in these two seaweeds. We conclude that certain seaweeds could be good sources of bioavailable iron. However, many seaweeds contained high levels of arsenic or other minerals which could limit regular consumption as a safe source of iron.

1. Introduction

Seaweeds have long been known to contain high levels of iron and other minerals, with many exhibiting higher levels of iron than terrestrial plants (Rupérez, 2002; MacArtain et al., 2007). However, the bioavailability of seaweed iron to humans is poorly understood.

Cultures that developed in coastal areas around the world have historically eaten seaweeds as a part of their diet (McHugh, 2003). Many consider seaweeds as nutritious foods, in part, for their potential to supply mineral nutrients to the body. Recently, there is growing interest by non-traditional consumers of seaweeds to incorporate these "sea vegetables" into their diets, or to use dried seaweeds as nutritional supplements. Many sea vegetable websites promote seaweeds as good sources of iron despite the lack of bioavailability studies (Whfoods.com, 2015; Seaveg.com, 2015; Vegkitchen.com, 2015). It would be useful to both traditional and non-traditional consumers of seaweeds to have valid information on seaweed iron bioavailability to help make informed decisions on the nutritional use of these foods. Additionally, identifying seaweeds with high iron bioavailability, and promoting their consumption, may be an economical way to help reduce the prevalence of iron deficiency.

Iron deficiency is a general term for suboptimal levels of iron in the body for health, cognitive function and physical activity. Deficiency symptoms can occur over a range of tissue iron levels and are thought to begin in conjunction with low levels of serum ferritin, progressing in severity to iron deficiency anemia (Brownlie et al., 2002; Zimmermann and Hurrell, 2007; Sawada et al., 2014). The prevalence of the various degrees of iron deficiency are not well known. The World Health Organization estimates that over 15% of the world's population has iron deficiency anemia (WHO, 2008). The prevalence of iron deficiency without anemia is unknown, but is probably considerable given the large number of people with iron deficiency anemia.

Abbreviations: DMEM, Dulbecco's modified Eagle's medium; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; ELISA, enzyme-linked immunosorbent assay; U.S. FDA, U.S. Food and Drug Administration; NLEA, Nutritional Labeling and Education Act; DV, Daily Value; RACC, reference amount customarily consumed; MRL, oral minimum risk level; WHO, World Health Organization.

* Corresponding author. Tel.: +1 808 956 3837; fax: +1 808 956 4024.
E-mail address: edu">mdunn@hawaii.?edu (M.A. Dunn).
[1] Present address: Food Science and Human Nutrition Department, University of Florida, Institute of Food and Agricultural Sciences, P.O. Box 110370, 359 FSHN Building, 572 Newell Dr., Gainesville, FL 32611-0370, USA.

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Iron deficiency is a public health problem in both underdeveloped and developed countries. It can be caused by a number of factors such as blood loss; malabsorption due to infections, parasites, or disease; low iron intake and/or iron bioavailability from the diet, or a combination of such factors. High risk populations are those that have a high requirement for iron such as growing children, pregnant and premenopausal women, and individuals with chronic blood loss or malabsorption.

Dietary strategies to reduce iron deficiency include food fortification, iron supplementation, and dietary diversification to incorporate iron-rich foods and foods with high iron bioavailability into the diet (WHO, 2014). If seaweeds are found to be good sources of bioavailable iron, they could be a part of dietary diversification strategies, especially in plant-based diets. Dried seaweeds could also be used to fortify other foods, or as a dietary supplement to help reduce the prevalence of iron deficiency.

The objectives of the study reported here were to evaluate mineral content and iron bioavailability in commonly consumed seaweeds. Iron bioavailability was assessed using the *in vitro* digestion/Caco-2 cell culture method of Glahn et al. (1998). Several studies have demonstrated the validity of this *in vitro* method as an index of iron bioavailability in humans, and have discussed its benefits and limitations (Au and Reddy, 2000; Sharp, 2005; Yun et al., 2004). This method is useful to compare iron bioavailability among a group of similar foods and identify ones that provide greater amounts of bioavailable iron per unit weight. The method does not directly quantify the amount of bioavailable iron in foods; rather it provides a relative index of iron bioavailability among the foods tested. We used this method to compare iron bioavailability in seaweeds to a reference food, cooked spinach. By including spinach we hoped to identify seaweeds that provide greater amounts of bioavailable iron than this well-studied, terrestrial, plant source of iron. We also used this *in vitro* method to test both the ability of vitamin C to enhance iron bioavailability from seaweeds, and the potential for seaweeds to inhibit iron bioavailability from ferrous sulfate. Results show that seaweeds vary greatly in iron bioavailability and its potential to be enhanced by vitamin C. We also present evidence that some seaweeds may inhibit the absorption of exogenous, non-heme iron.

2. Materials and methods

2.1. Chemicals, water and glassware

Unless otherwise stated, all chemicals were purchased from Sigma Chemical (St. Louis, MO). All glassware was soaked overnight in 10% HCl/10% HNO_3 and rinsed with deionized water before use. All water used in the experiments was cell culture grade, deionized water.

2.2. Sample collection

Thirteen seaweed samples were obtained for mineral analysis prior to selecting a subset for iron bioavailability experiments. The 13 seaweeds are characterized in Table 1 and include: aonori, dulse (powder and flake), gorilla ogo, green ogo, hijiki, limu wawae'iole, nori, red ogo, rockweed, sea lettuce (powder and flake), and wakame. Seaweeds were obtained in the fresh or dried state from either local markets in Honolulu, HI or online from Maine Coast Sea Vegetables Franklin, ME (Seaveg.com, 2015). Spinach was obtained fresh from a local grocery store in Honolulu, HI. Fresh seaweeds and spinach were processed within 24 h of purchase as described below.

2.3. Sample preparation

Fresh spinach: stalks and stems were removed; the leaves were weighed, rinsed with deionized water and pat dry. Leaves were then boiled for 10 min in a stainless steel pot using deionized water, and placed on ice to prevent further cooking. The spinach was then drained for 5 min to remove any residual water and homogenized in a Cuisinart food processor with stainless steel blades. The homogenized spinach was put into Teflon weigh boats, lyophilized (VirTis Virtual 50xl, SP Scientific), sealed in plastic tubes, and kept in a desiccator at room temperature until analyzed for mineral content and iron bioavailability.

Seaweed samples: within 24 h of purchasing, fresh seaweeds were thoroughly washed three times in deionized water, and any visible invertebrates, sand or debris were removed. The washed seaweed samples were spun in a salad spinner for 30 s to remove excess water and weighed. Then, both the washed seaweed samples and the seaweed products purchased in the dry form were dried to a constant weight on acid-washed, Pyrex plates at 50 °C in a stainless-steel drying oven. The dried samples were then ground into a fine powder using a coffee grinder with stainless steel bowl and blade. The dried, ground, samples were sealed in plastic tubes and kept in a desiccator at room temperature until analyzed for mineral content and iron bioavailability.

2.4. Mineral analysis

Three, 2-g aliquots of each dried, ground, sample were sent to the Louisiana State University Agricultural Center for mineral analysis by inductively coupled plasma-emission spectroscopy (ICP-ES) after wet digestion and dilution in deionized water (LSU, 2015). The limit of detection for theses analyses was the mineral concentrations of the lowest ICP-ES calibration standard. Seventeen minerals were measured: aluminum, arsenic, boron, calcium, copper, lead, iron, magnesium, manganese, molybdenum, nickel, phosphorus, potassium, selenium, sodium, sulfur and zinc. National Institutes of Standards and Technology (Gaithersburg,

Table 1
Seaweeds analyzed for mineral content.

Common name(s)	Classification	Scientific name	Geographic origin	Sample state at purchase
Aonori, Green laver	Green algae	*Enteromorpha* spp.	Japan	Dry flakes
Dulse	Red algae	*Palmaria palmata*	Maine	Dry powder and flakes
Gorilla ogo	Red algae	*Gracilaria salicornia*	Hawaii	Fresh
Green ogo	Red algae	*Gracilaria parvispora*	Hawaii	Fresh
Hijiki	Brown algae	*Hizikia fusiformis*	Japan	Dry strips
Limu wawae'iole	Green algae	*Codium edule*	Hawaii	Fresh
Nori, Laver	Red algae	*Porphyra* spp. (*P. yezoensis, P. tenera vietnamensis*)	China	Dry sheets
Red ogo	Red algae	*Gracilaria coronopifolia*	Hawaii	Fresh
Rockweed, Egg wrack	Brown algae	*Ascophylum nodosum*	Maine	Dry powder
Sea lettuce	Green algae	*Ulva lactuca*	Maine	Dry powder and flakes
Wakame	Brown algae	*Undaria pinnatifida*	Japan	Dry strips