EVALUATION OF SEAWEEDS AS FOOD SOURCES OF IRON:
MINERAL CONTENT AND IRON BIOAVAILABILITY USING
THE IN VITRO DIGESTION/ CACO-2 CELL METHOD

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ACKNOWLEDGEMENT

After four years of independent living, I begrudgingly returned to my childhood home in Hawai‘i in 2011, dismal, naive and apprehensive to begin the graduate school chapter of my life. This opportunity was thrown at me and I kind of just took it and ran with it, never really expecting what would come of it. Now, as I embark on the last leg of my academic journey, I leave Hawai‘i feeling bittersweet but prepared.

I’d first like to acknowledge my thesis committee, Dr. Dobbs and Dr. Titchenal. I cannot give back the hours they spent helping me write this thesis, but because of them I am a better researcher and I only hope to make them proud in the future. I would also like to send a heartfelt thank you to my advisor Dr. Dunn. Research is an extremely frustrating and arduous task, but Dr. Dunn’s patience with me, understanding of my mistakes and genuine love for the science serve as my inspiration to continue in this field.

I leave UH Manoa with a lifetime of new friends who have become my family and I am forever grateful for the laughs, advice, and good times we shared. Lastly, my mom has been my strength, truly defining a mother with her enduring kindness and support. She may not know the difference between biology and chemistry, but her undying belief in me is all I’ve ever needed. And for that, no words can describe my appreciation. My research was funded by USDA Hatch award #279H.
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LIST OF ABBREVIATIONS

ANOVA Analysis of Variance
AMDR Acceptable Macronutrient Distribution Ranges
Caco-2 Human colonic adenocarcinoma cells
CDC Center for Disease Control
CO2 Carbon dioxide
DHHS Department of Health and Human Services
DW Dry weight
DMEM Dulbecco’s modified eagle medium
DMT1 Divalent metal transporter-1
DNA Deoxyribonucleic acid
DRI Dietary reference intakes
DV Daily Value
EDTA Ethylenediaminetetraacetic acid
FAO Food and Agriculture Organization
FBS Fetal bovine serum
FDA Food and Drug Administration
Fe Iron
Fe2+ Ferrous iron
Fe3+ Ferric iron
FeSO4 Ferrous sulfate
FW Fresh weight
H2SO4 Sulfuric acid
H2O2 Hydrogen peroxide
HEPES 4-(2-hydroxyethyl)-1-piperazinethanesulfonic acid
HBBS Hank’s balanced salt solution
HCl Hydrochloric acid
HCP-1 Heme carrier protein-1
HO Heme oxygenase-1
HNO3 Nitric acid
ICP-ES Inductively coupled plasma-emission spectroscopy
KCl Potassium chloride
MEM Minimal essential media
MW Molecular weight
NaHCO3 Sodium bicarbonate
NaCl Sodium chloride
NLEA Nutrition Labeling and Education Act
PPM Parts per million
RACC Recommended Amount commonly consumed per eating occasion
RDA Recommended Dietary Allowance
Tf Transferrin
USDA United States Department of Agriculture
WHO World Health Organization
ABSTRACT

Iron (Fe) deficiency is the most common nutrient disorder in the U.S. and worldwide, affecting nearly 2 billion people (WHO, 2008). Often Fe deficiency is attributed to poor Fe bioavailability in foods, especially in plant-based diets. With the U.S. Dietary Guidelines suggesting a reduced intake of meat and increased intake in fresh produce and whole grains, the prevalence of Fe deficiency could increase. Thus, there exists a need to discover novel plant foods that supply bioavailable Fe to the diet. Seaweeds have been shown to be rich sources of Fe and are grown and commonly eaten in Hawai’i. The purpose of this study was to evaluate the mineral content and Fe bioavailability of various seaweeds to determine their potential nutritive value as a good source of Fe. In this study the mineral content of eleven common and Hawaiian seaweeds (Aonoriko, Dulse, Gorilla Ogo, Green Ogo, Hijiki, Nori, Red Ogo, Rockweed, Sea Lettuce, Wakame, and Wawae’iole) was determined via inductively coupled plasma mass spectrometry (ICP-MS). Of the eleven seaweeds, six (Nori, Red Ogo, Rockweed, Sea Lettuce, Wakame, and Wawaei’ole) were selected on the basis of popularity in western diets, total iron content, and native Hawaiian origin for further analysis of their Fe bioavailability relative to spinach. Relative Fe bioavailability was assessed using a Caco-2 cell/ in vitro digestion method. Results showed no significant difference in bioavailable iron between spinach and Rockweed, Wakame, or Wawaei’ole. Similarly, while Red Ogo contained five times as much Fe as spinach, the bioavailability of that Fe was low even when enhanced with Vitamin C. In contrast, Nori and Sea Lettuce provided 2-4 times more bioavailable Fe per gram dry weight than spinach. When supplemented with vitamin C, an enhancer of Fe absorption, all seaweeds showed a significant increase in Fe bioavailability as expected (except Red Ogo). This suggests that variation between seaweeds may stem from
different contents of enhancers (Vit C) and inhibitors (possibly polyphenols or manganese) in the seaweeds themselves. We conclude that Nori and Sea Lettuce provide more bioavailable Fe than spinach and thus should be considered good sources of dietary Fe. Red Ogo is a poor source of Fe possibly due to either high polyphenol or manganese content. It remains to be determined if seaweeds can be recommended in practical amounts to improve Fe status, and if increased consumption will raise concerns for excessive intakes of potentially toxic minerals such as iodine.
STATEMENT OF PROBLEM

Despite the implementation of large-scale nutritional intervention programs, iron deficiency remains a public health problem in most developed countries (WHO 2008). Typically iron deficiency can be attributed to either 1) increased iron needs via blood loss, rapid growth or pregnancy or 2) decreased dietary iron intake or absorption (Center for Disease Control and Prevention, 1998). The latter is typically due to overall low iron intake or low food bioavailability. Attempts are being made to find effective and realistic food-based strategies that increase iron intake to help alleviate iron deficiency. Dietary iron bioavailability is low in populations that consume plant-based diets. In addition, as the 2010 Dietary Guidelines promote the consumption of plant foods (USDA and DHHS, 2010) there is also an expected increase in inhibitors of iron absorption. Thus there is a need to find novel plant foods that provide bioavailable iron to the diet.

Seaweeds represent an abundant, and natural plant resource for nutritional use with potential health benefits and economic value in aquaculture (Fleurence et al., 2012). Taking into account the prevalence of iron deficiency and the prospective importance of marine food for human nutrition, studies regarding nutrient and mineral bioavailability are needed for assessing the mineral toxicity risk and real nutritional value of these foods.
CHAPTER 1: LITERATURE REVIEW

IMPORTANCE OF IRON

Functions

Iron is the most abundant trace element in humans and its functions in the body are numerous. This mineral is involved in a myriad of vital physiological processes and is found most frequently as components of proteins, including cofactors for enzymes (Bothwell et al., 1979). An important role of iron is its contribution to heme proteins. The iron atom in the center of heme enables the molecule to 1) store and transport oxygen in muscles and other tissues (hemoglobin and myoglobin) and 2) to transport electrons through the respiratory chain (cytochromes) (FAO/WHO, 2005).

In addition to its role in heme, iron is an important component for at least 200 cellular enzymes necessary for normal body functions. These functions include such vital processes as energy metabolism, DNA synthesis, cell growth and differentiation, and gene regulation (FAO/WHO 2005).

Heme vs. Non-heme Iron

Of iron’s several oxidation states (Fe$^{6+}$ to Fe$^{2-}$), only the Fe$^{2+}$ (ferrous iron) and Fe$^{3+}$ (ferric iron) forms constitute dietary iron. Ferrous and ferric iron forms remain stable in the aqueous environment of the human body (Collins and Anderson, 2012). Dietary iron can be further classified into heme and non-heme iron, which are absorbed by two different mechanisms in the small intestine.

Heme iron is associated with the proteins hemoglobin and myoglobin and is found primarily in red meat and organ products. This form of iron has a greater bioavailability than non-heme iron with an estimated absorption of 20-30%. Because heme iron is more
effectively absorbed, it accounts for more than half of the actual iron absorption by the body (Aspuru et al., 2011). Despite this fact, heme iron does not contribute significantly to the typical iron content in the U.S. diet, making up only an estimated 10-16% of total dietary iron consumption (Carpenter and Mahoney 1992).

Non-heme iron is found in a variety of foods, and is the dominant form of iron consumed in a typical western diet (Aspuru et al., 2011). This type of iron is abundant in plant foods (i.e. grains, legumes and vegetables). Although non-heme iron is poorly absorbed, with an estimated absorption rate of 1-4% (Zimmermann et al., 2005), non-heme iron still makes a significant contribution to total dietary iron absorbed.

**Digestion & Absorption Mechanisms**

Iron absorption (Figure 1.1) occurs primarily in the enterocytes of the upper portion of the small intestine, or duodenum (Benito & Miller, 1998). The structure of the small intestine is designed to maximize surface area and thus maximize nutrient absorption (Collins and Anderson, 2012). Finger-like projections, called villi, project into the lumen of the gastrointestinal tract and consist of enterocytes with an apical side in contact with the lumen of the gastrointestinal tract, and a basolateral side in contact with the bloodstream. The apical side of the enterocytes is covered in tiny hair-like microvilli that extend into the intestinal lumen. These projections are referred to as the apical “brush border” and house various digestive enzymes (Collins and Anderson, 2012). To be used by the body, iron and other nutrients, must be taken up by the enterocyte, transported through the cell, and released through the basolateral membrane into the bloodstream (Benito & Miller, 1998; Polin et al., 2013).
Heme iron and non-heme iron are absorbed by different mechanisms due to their varying chemical structures (Anderson et al., 2012). The exact mechanism of heme iron absorption is not well understood, but its ability to remain soluble in the aqueous intestinal solution, allows it to be taken up easily (Miret et al., 2003). Heme carrier protein-1 (HCP1)/proton-coupled folate transporter has been identified as an apical heme transporter on the brush border that imports heme iron into the enterocyte (Gunshin et al., 2005; Anderson et al., 2012). Once within the enterocyte, ferrous iron is released from heme by heme oxygenase (HO) (Miret et al., 2003; Anderson et al., 2012).

Non-heme iron is found in plant foods (vegetables, grains and legumes) and is usually bound to other food components (Anderson et al., 2012). Before absorption can occur, non-heme iron must be released from these components by the acidic gastric juice and proteases of the stomach and is mostly present as ferric iron (Fe$^{3+}$) (Miret et al., 2003). Ferric iron, however, is insoluble in the alkaline environment of the duodenum and is subsequently reduced to the ferrous form by ferric reductase activity on the brush border (Anderson et al., 2012). Various ferroreductases have been identified to participate in this reduction step, including duodenal cytochrome B (Dcytb) (McKie 2008). Ferrous iron may then be imported into the enterocyte by various transport proteins within the brush border such as ferrous iron transporter, divalent metal iron transporter 1 (DMT1) (McKie 2008; Collins and Anderson 2012). McKie (2008) showed that mice with intestinal-specific excision of DMT1, died shortly after birth due to severe anemia, implying the singular importance of this transporter.
Iron Transport, Storage and Export

Once imported into the enterocyte, iron will have been (Anderson and Vulpe 2009):

1) Exported out of the enterocyte through the basolateral membrane to enter circulation for intra-tissue transport
2) Stored in the enterocyte for future use, or
3) Utilized by the enterocyte for various intracellular functions.

A multi-pass trans-membrane protein, Ferroportin (Fpn1), is responsible for the movement of iron out of the enterocyte and into the bloodstream (Miret et al., 2003; Anderson et al., 2012). Currently, it is the only known intestinal iron export protein in humans (Anderson et al., 2012). Once ferrous iron passes through Fpn1 it undergoes reduction to the ferric form via hephaestin (Hp) where it then binds to the transport protein Transferrin (Tf) (Anderson and Vulpe, 2009).

If not immediately utilized, iron in the enterocyte is believed to enter a common cytosolic iron pool for storage (Collins and Anderson, 2012), however the nature of this pool remains poorly understood. Circulatory iron not needed by the body is transported by Tf to
three main storage sites; the liver (60%), bone marrow, or spleen (40%) (Anderson and Vulpe, 2009). Ferritin (Ft) is the primary storage form of iron in cells (Anderson and Vulpe, 2009).

Currently there are no known regulatory mechanisms for the excretion of iron (Collins and Anderson, 2012). However, daily losses occur due to the shedding of intestinal and other epithelial cells, menstruation, urine and other sources such as sweat and hair. Premenopausal women can lose up to 2 mg/day due to losses from menses (Carpenter and Mahoney 1992). Classified as an essential nutrient, the human body is incapable of synthesizing iron and thus losses must be compensated exclusively through dietary intake. When dietary intake is insufficient, body iron stores decrease and iron homeostasis is interrupted. Unless body needs are replenished, iron deficiency develops (FAO/WHO 2005).

**ii. DIETARY IRON RECOMMENDATIONS**

Individual iron needs are dependent on several factors including age, gender, life-stage (e.g. pregnancy and infancy), diet etc (FAO/WHO 2005). Yet there are general recommendations, the Recommended Dietary Allowances (RDAs), for healthy individuals based on age, gender, and diet pattern (Table 1-1).

**TABLE 1-1.** Recommended Dietary Allowance (RDA) for iron by age and sex (Food and Nutrition Board, 2006)

<table>
<thead>
<tr>
<th>Group</th>
<th>Age</th>
<th>Iron (mg/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infants</td>
<td>7-12 months</td>
<td>11</td>
</tr>
<tr>
<td>Adolescents</td>
<td>14-18 years</td>
<td>11 (males); 15 (females)</td>
</tr>
<tr>
<td>Males</td>
<td>19-70 years</td>
<td>8</td>
</tr>
<tr>
<td>Females</td>
<td>19-50 years</td>
<td>18</td>
</tr>
</tbody>
</table>
As seen in the table above, iron needs increase during periods of blood loss such as menstruation in females or periods of rapid growth such as infancy and pregnancy (to account for the growth of the fetus and subsequent increase in blood volume) (Scholl, 2005). These values, established by the Food and Nutrition Board of the Institute of Medicine, represent the average daily dietary intake that is sufficient to meet the nutrient requirements of 97% of healthy individuals (Food and Nutrition Board, 2006). Failure to meet this RDA can result in disruptions in iron status and may ultimately lead to iron deficiency.

The aforementioned RDAs are tailored for omnivorous diets that obtain at least 10% of their dietary iron from heme or meat sources. Consequently these values do not hold true for vegetarian diets, whose iron is derived from non-heme iron (Otten et al., 2006). A comparison between omnivorous and vegetarian diets reveals a 10% difference in iron bioavailability. To account for this difference in absorption, the RDAs for vegetarian diets are increased 2 fold (1.8x) (Otten et al., 2006). This raises the RDA to 14 mg/d for adult men and 33 mg/d for adult, non-pregnant women.

Iron is found in a wide variety of commonly consumed foods and food ingredients. In the 25th release of the National Nutrient Database nutrient list for iron sources (USDA National Nutrient Database for Standard Reference), selected foods are listed in order of descending iron content expressed in mg of iron per reference amount of each food. Table 1-2 provides a list of commonly eaten foods and iron content per common measure.
### TABLE 1-2. Iron content of common foods

<table>
<thead>
<tr>
<th>Food</th>
<th>Weight (g)</th>
<th>Iron content (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ready-to-eat cereals, fortified cereals; varied, ~ 1 cup</td>
<td>~ 35 (average)</td>
<td>6.68 – 18.70</td>
</tr>
<tr>
<td>Soybeans, boiled; 1 cup</td>
<td>172</td>
<td>8.84</td>
</tr>
<tr>
<td>Lentils, mature &amp; boiled; 1 cup</td>
<td>198</td>
<td>6.59</td>
</tr>
<tr>
<td>Spinach, cooked &amp; boiled with no salt; 1 cup</td>
<td>180</td>
<td>6.43</td>
</tr>
<tr>
<td>Cooked beef liver; 3 oz</td>
<td>85</td>
<td>5.24</td>
</tr>
<tr>
<td>Turkey giblets, simmered; 1 cup</td>
<td>145</td>
<td>4.92</td>
</tr>
<tr>
<td>Beans, baked, canned; 1 cup</td>
<td>254</td>
<td>3.02</td>
</tr>
<tr>
<td>Chicken liver, cooked; 1 liver</td>
<td>19.6</td>
<td>2.28</td>
</tr>
<tr>
<td>Canned clams; 1.5 cup</td>
<td>85</td>
<td>2.28</td>
</tr>
<tr>
<td>Cooked ground beef, 85% lean; 3 oz</td>
<td>85</td>
<td>2.21</td>
</tr>
</tbody>
</table>


In light of different nutrient needs between age groups, gender, nutritional status, etc., the dissemination of the appropriate nutrition content to consumers is important. Access to the nutritive value of a food as a good or poor source of a particular nutrient can help consumers make informed food choices to sustain adequate nutrient levels. The Daily Values (DV) were developed by the Food and Drug Administration (FDA) to help consumers determine the nutrient level in a serving of food in relation to their approximate dietary requirement (Otten et al., 2006). All packaged or prepared food products require nutritional labeling through the Nutrition Labeling and Education Act (NLEA) [Title 21 Code of Federal Regulations (21 CFR)]. This does not apply to fresh produce unless a nutrient claim is made otherwise claiming the food as a ‘good source’ or ‘excellent source’ of a nutrient (CFR § 101.9, 101.42). A nutrient content claim for a ‘good’ source translates to a food item that contains 10-19% of the DV per Reference Amounts Customarily Consumed per eating occasion (RACC) (CFR § 101.12-13). A claim for ‘excellent’ is 20% or more of the DV per RACC. The RACC was
established by the FDA using data from national food consumption surveys. These amounts are defined as the average amount of a given food customarily consumed at a single sitting and their primary function is in nutrition labeling (CFR § 101.12). For example, reference amounts for cooked vegetables are listed as 85 grams per serving and the DV for iron is 18 mg/day. Using this designation, a selected vegetable would need to meet 1.8-3.6 mg of iron (10-20%) per 85 gram serving to be considered a good source of iron. A vegetable excellent in iron would contain greater than 3.6 mg iron (20%) per RACC.

### iii. IRON DEFICIENCY

Iron deficiency is the most common nutrient deficiency in the world, affecting 30-40% of the global population (WHO 2008). This nutrient deficiency affects women and children at epidemic proportions in both industrialized and developing countries. In the United States (U.S.) alone, 9-11% of women of childbearing age are iron deficient (Perry et al., 1995; WHO 2008). This frequency is doubled in poorer, less educated, and minority populations (Looker et al., 1997). Also at risk, are pregnant women of low-income areas in the US, where the frequency of iron deficiency anemia (IDA) in the first, second, and third trimesters is 2%, 8%, and 27% respectively (Perry et al., 1995).

Iron deficiency is a condition that occurs when physiological requirements are not met by iron absorption from the diet. It is a result of increased iron needs, low dietary intake, inadequate absorption, or iron losses (CDC 2002). Iron deficiency causes vary depending on age, gender, socioeconomic status etc.

Deficiency levels range from depleted iron stores without functional impairment, to a more severe condition of iron deficiency anemia (Center for Disease Control and Prevention,
Additional risk factors for iron deficiency include vegetarian diets, adolescence, and menstruation and pregnancy in women. (Zimmermann and Hurrell, 2007). Consequently, those particularly at risk include women and children of developing countries where plant-based diets provide low levels of bioavailable iron. Common symptoms of iron deficiency include decreased productivity and work performance, impaired cognitive development, changes in immune function, and increased risks of preterm delivery, infant mortality and low birth weight (Scrimshaw, 1984; Brabin et al., 2001; Hass and Brownlie, 2001). The continuing increase in iron deficiency prevalence has also incurred considerable economic costs as a result of these cognitive and functional health consequences (Horton and Ross, 2003).

**Iron Bioavailability**

Habitual consumption of a diet low in iron bioavailability is a major contributor to iron deficiency (Heath & Fairweather-tait, 2002; Zimmermann et al., 2005). Iron bioavailability is defined as the extent to which iron is absorbed from the diet and used for normal human physiological functions (Watzke 1998; Hurrell and Egli 2010). Bioavailability is an important concept when considering mineral nutrition but is not directly correlated to total mineral content. Rather, bioavailability is a combination of several different dietary and physiological factors including the amounts of non-heme and heme iron in the diet, dietary inhibitors and enhancers that influence iron bioavailability, and the iron status of the individual (Fairweather-Tait 1993; Heath and Fairweather-Tait, 2002).

There exist two types of dietary iron: non-heme iron found in both animal and plant foods and heme iron found in the hemoglobin and myoglobin of animal sources (Anderson
and Vulpe, 2009). In meat, 30-70% of the total iron is heme iron, of which 15-35% is absorbed (Carpenter and Mahoney, 1992). Dietary iron in plants is non-heme iron and its absorption is often less than 10% (Hunt, 2002; Zimmerman et al., 2005). Consequently, dietary iron bioavailability is low in populations consuming habitual plant-based diets with little meat (Heath & Fairweather-tait, 2002). One major reason for this discrepancy is due to the difference in iron bioavailability between vegetables and animal sources. In vegetarian diets, the non-heme form of iron, in combination with the inhibitory components found in vegetables, limits the amount of iron absorbed (Zimmerman et al., 2005; Zimmerman and Hurrell, 2007). Phytates, polyphenols, and calcium are the primary inhibitors of iron absorption and are common components in plant sources (Hallberg et al., 1993). However, the absorption of non-heme iron can be increased by simultaneous meat or ascorbic acid consumption (Taylor et al., 1986). Meat provides iron in the more bioavailable heme form and is thus unaffected by these inhibitory components.

**Factors that Affect Bioavailability**

**Physiological Factors**

Iron absorption and utilization is dependent on the individual physiological needs of the body. The literature has shown an inverse relationship between innate iron status and iron absorption (mainly non-heme iron absorption) (Carpenter and Mahoney, 1992). Inter-individual variations in iron status present problems when trying to administer iron test meals in research. Variability that arises from individual iron status makes it difficult to determine whether differences in absorption are due to the iron status of the subject or properties of the meal (Hallberg, 1981; Wienk et al., 1999). Pregnancy also is associated with an increase in
the bioavailability of dietary iron (Scholl, 2005; Brabin et al., 2001).

Other host factors such as infection/inflammation, obesity, additional nutritional deficiencies, or disease conditions may also negatively influence iron absorption and metabolism (Hallberg, 1981).

**Dietary factors**

Various dietary factors have an effect on the bioavailability of iron and other minerals. Meals with similar iron contents but dissimilar compositions have shown tenfold differences in bioavailability (Hallberg & Hulthen, 2000). This suggests that different factors present in foods affect iron absorption by either enhancing or inhibiting its uptake into the enterocyte. For example, vegetarian diets are capable of containing amounts of iron equal to omnivorous diets (Crag, 1994). However, the non-heme iron from a plant-based diet is less bioavailable due to differences in food structure and the accompanying presence of various enhancers and inhibitors in plant foods. Modifiers of non-heme iron absorption commonly found in foods are listed below in Table 1.3.

Table 1.3- Dietary factors affecting non-heme iron absorption (adapted from Hallberg and Hulthen, 2000; Zimmerman, 2005; Wessling-Resnick, 2014).

<table>
<thead>
<tr>
<th>Enhancers</th>
<th>Inhibitors</th>
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<tr>
<td>Ascorbic Acid</td>
<td>Calcium</td>
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<td>Histidine</td>
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<td>Zinc</td>
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**Inhibitors of iron absorption**

Several compounds are capable of binding with iron to either inhibit or enhance its absorption. Non-heme iron is particularly susceptible to inhibition by other dietary components. These inhibitory components can decrease absorption and include phytate and polyphenols in plants. Heme iron is not affected by these inhibitors and is thus considered to be more bioavailable than non-heme iron.

**Phytate**

Phytate, also called phytic acid or myoinositol hexaphosphate, is present in legumes, whole grains, and corn and inhibits non-heme iron absorption (Torre et al., 1991; Hurrell and Egli, 2010). The inhibitory effect of phytate can be attributed to the presence of negatively charged oxygen atoms within phosphate groups (Figure 1.2). Positively charged iron ions (and other mineral ions) bind between two negatively charged phosphate groups within phytate molecules forming large, insoluble iron-phytate complexes (Torre et al., 1991). Small amounts of phytate (5 to 10 mg) can reduce non-heme iron absorption by 50% (Reddy and Sathe, 2002). Also in a study done by Hallberg et al (1987), phytate was shown to affect iron absorption in a dose dependent manner.

![Fig.1.2 – The binding of iron by phytate](image)

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**Fig.1.2 – The binding of iron by phytate**
Despite inhibition of iron absorption by phytate, studies have suggested that simultaneous consumption of phytate-rich foods with dietary enhancers of absorption have a partial reversible effect on inhibition (Hallberg et al., 1989; Troesch et al., 2009). In their study, Hallberg et al (1989) used phytic acid phosphorus in a dose dependent manner to determine the effect of phytate on iron absorption. Although phytate significantly hindered iron absorption, this inhibition was neutralized with the addition of step-wise addition of vitamin C (Vit C). The authors showed that the overall neutralizing effect was related to the amount of phytate present, meaning more Vit C was needed to overcome a higher amount of phytate. Approximately 80 mg of Vit C was needed to fully reverse the inhibitory effect of 25 mg phytate and several hundred milligrams of Vit C was needed to offset the inhibitory effect of 250 mg phytate. Therefore, diets containing high amounts of phytate should also include high amounts of Vit C in order to counteract inhibition and maximize iron absorption.

*Polyphenols*

Polyphenols found in various fruits, vegetables, coffee, tea, wines, and spices, have been shown to markedly inhibit the absorption of non-heme iron (Hallberg, 1991; Hurrell and Egli, 2010). Many studies have looked at the inhibitory effect of polyphenols in different foods. Gillooly et. al. (1983) showed a significant inverse correlation between iron absorption and polyphenol content in various vegetables. The inhibitory effect of polyphenols is attributed to their ability to bind with iron and form large insoluble complexes in the gastrointestinal lumen, therefore, limiting uptake of iron at the brush border (Brune et al. 1989).
Flavonoids are an example of a class of polyphenols present in many different foods and that affect iron absorption (Tuntawiroon et al. 1991). Large flavonoid polymers, or tannins, occur in plants and are the polyphenols most associated with iron inhibition. Brune et al. (1989) compared the effect of three isolated tannins on iron absorption, (catechin, gallic acid, and chlorogenic acid) using an extrinsic tag method. Of the three, gallic acid was found to be the strongest inhibitor against iron absorption. This effect was thought to be due to the presence of trihydroxybenzene (galloyl) groups. Each group may bind with positively charged iron ions in the neutral pH of the duodenum forming an insoluble complex and preventing iron import into the enterocyte (Tuntawiroon et al., 1991). Further studies by the same author revealed a dose-dependent manner of this relationship (Tuntawiroon et. al., 1991). A similar dose-dependency was observed in an in vivo study that investigated the effect of stepwise addition of tannins on iron bioavailability in Indian women (Siegenberg et al., 1991).

Tannins and iron bioavailability have been studied in vitro using tannic acid. A study by Glahn et al (2008) looked at the in vitro effects of tannic acid on iron uptake using different iron sources (FeSO₄, FeCl₃ and FeEDTA) and Caco-2 cells with cell ferritin as a measure of uptake. The results demonstrated a marked decrease in ferritin formation from a iron:tannic acid ratio of 1:1 to 1:10. This provides further evidence for the chelating ability of tannins, regardless of iron source.

Other dietary factors consumed simultaneously with tannins have been shown to have an influence on the magnitude of iron inhibition. In an in vivo study, Kim and Miller (2004) noted that the overall effect of tea on iron absorption was inhibitory, but that the magnitude was dependent on the duration of tea ingestion. Rats exposed to long-term tea ingestion showed a significantly greater absorption of iron compared to those of short-term tea
ingestion (Kim and Miller, 2004). Similarly, researchers found that the inhibitory effect of tannic acid was strongest when consumed in conjunction with iron and persisted (albeit at a weaker level) until 1 hour after non-heme iron intake (Disler et al. 1975).

Similar to phytate, polyphenolic iron inhibition can be offset by the concurrent consumption of vitamin C. Studies (Siegenberg et al., 1991; Tuntawiroon et al., 1991) have shown a reversal of inhibition in a dose dependent manner with as little as 50 mg of Vit C showing a significant improvement in iron absorption. However, this neutralization is only possible when Vit C is consumed before the addition of tannins (South and Miller, 1998). According to the authors, it is imperative to avoid competitive binding thereby 1) allowing Vit C to bind to iron and make it unavailable to tannins or 2) reducing ferric iron to ferrous iron thus increasing solubility.

In summary polyphenols can negatively affect the absorption of dietary iron. While phenolic inhibition can be potent, its magnitude varies with amount and can be partially offset by simultaneous consumption of dietary enhancers such as Vit C.

Other minerals

Several elements when ingested with iron can reduce non-heme iron absorption either through competitive binding, or chelation (Zimmerman and Hurrell, 2007).

The interaction of zinc and iron may negatively affect each other’s absorption, although studies are contradictory and remain inconclusive. Although the mechanism for this inhibition is unclear, it is purported that the two minerals compete for the same intestinal transporters (mainly DMT1) because of their similar chemical charges (Olivares et al., 2007).
There also appears to be a negative interaction between calcium and iron, though the mechanism remains poorly understood (Hallberg et al., 1993). This inhibition is seen to affect both heme and non-heme iron and is thus suggested to occur within the intestinal cell itself (Hallberg et al. 1993). In a single meal study by Hallberg et al (1991), the addition of 150 mg of calcium to wheat bread rolls, reduced iron absorption by about 50%. Similar reductions in iron absorption have also been observed with milk consumption (Hallberg and Rossander, 1991). One hypothesis is that inhibition occurs via chelation of the two minerals with phosphorus. This renders the iron insoluble and unable to be transported through the intestinal brush border. Alternately, calcium may inhibit iron absorption within the enterocyte by disrupting iron transport to the basolateral membrane. However, other studies have shown no effect on absorption with calcium supplementation or calcium-rich sources such as milk (especially when eaten as a composite meal) (Hallberg and Rossander 1982). The minimum amount of calcium needed to exert an inhibitory effect has not been established, but no effect was seen when less than 40mg of calcium was added to a meal. The saturation level appears to be 300 mg; intake exceeding this amount does not promote further inhibition (Hallberg et al., 1991). More research regarding the relationship between calcium and iron is needed.

Iron and copper appear to share an interrelationship because of the role of copper-containing proteins hephaestin and cerruloplasmin in iron metabolism (Anderson and Vulpe, 2009). These enzymes are responsible for ferrous iron oxidation and transferrin mobilization. The inverse relationship between copper intake and iron absorption has been attributed to a possible competitive binding for import into the cell via DMT1. In theory, copper reduction would impair iron reductase activity, and thus iron would not be mobilized out of enterocytes, ultimately leading to iron deficiency.
These findings suggest that minerals with chemical similarities to iron can compete for transport proteins or other uptake mechanisms, as well as form chelation compounds that may facilitate or hinder absorption. The consequences of these interactions may depend on the relative concentrations of the minerals or meal administration may also have an effect. For example, minerals in the presence of a composite meal may exert a lesser negative influence on iron absorption versus when given in an aqueous solution. These factors should be taken into consideration when evaluating the inhibitory effects of other minerals on iron and their interactions.

**Summary**

In summary, there are several classes of compounds found in different concentrations within foods that are known to inhibit iron absorption. Iron bioavailability is the outcome of the inherent composition in foods of these inhibitors and enhancers. In addition particular effects appear to be independent of, rather than additive to, other effects displayed by dietary components. This fact makes determination of absolute bioavailability of non-heme iron very difficult. As a result, iron bioavailability can only be defined in relative terms of the overall interaction between dietary inhibitors and enhancers, which ultimately determines non-heme iron bioavailability from the whole diet. Awareness of these interactions, along with a knowledge of dietary iron intake and enhancers and inhibitors of a population could help implement strategies to improve iron status. (Sandstrom, 2001). Taken together, these findings could have significant nutritional implications, as the present trend in Western countries of increasing fruit, vegetable and whole grain intake will also increase the dietary intake of many iron inhibitors.
Enhancers of iron absorption

Vitamin C

Some dietary factors such as Vitamin C (also known as ascorbic acid) and meat have been found to enhance non-heme iron absorption (Hallberg et al., 1979). Vitamin C (Vit C) is a particularly potent enhancer for non-heme iron absorption (Hallberg et al., 1989). The enhancing properties of Vit C are often attributed to its capabilities as a reducing agent (Wessling-Ressnick, 2014). In the initial acidic pH of the duodenum, Vit C is thought to assist the reductases of the apical brush border in reducing ferric iron to the more soluble ferrous form (Hallberg et al., 1989) thus improving overall intestinal iron absorption by DMT1.

Vitamin C has shown a dose-dependent enhancement on non-heme iron absorption in foods and/or composite meals when simultaneously consumed with lemonade (Olivares et al., 2007), papaya juice (Layrisse et al., 1974), and orange juice (Rossander et al., 1979).

Vitamin C, when consumed as part of a single meal, can also act as a neutralizing compound by counteracting the inhibitory effect of various dietary inhibitors on non-heme iron absorption (Rossander et al., 1979; Hallberg et al., 1989). Thus the iron absorption from vegetarian meals can be best optimized by the inclusion of vitamin C-containing vegetables.

Meat Factor

Various animal tissues enhance the bioavailability of both heme and non-heme iron (Taylor et al., 1986; Hallberg et al, 1979). This effect is referred to as the “meat” factor (Cook and Monsen 1976; Huh 2004). Previous studies have shown about 2-3 fold increases in iron absorption with meat (Taylor et al., 1986; Hallberg et al, 1979).
Taylor et al (1986) used extrinsic labeling of heme and non-heme iron in different foods to observe the enhancing effect of meat on iron absorption from corn and black beans. The authors found that absorption doubled with the addition of veal. Later studies found similar results with other meat sources such as chicken, pork, beef, and fish (Layrisse et al., 1974; Martinez-Torres 1975; Cook and Monsen, 1976).

The enhancement mechanisms of the meat factor remain to be elucidated. The individual constituents responsible for the enhancing effect of meat on intestinal iron absorption are also unclear. It is proposed that the digested peptide products from animal tissues may serve as chelators for non-heme iron forming complexes that increase solubility and thus, facilitate absorption (Hurrell et. al 1988). Hallberg et al. (1979) stated that specific peptides formed during the digestion of meat may contribute to the meat factor either by acting as a carrier of heme and non-heme iron or by preventing the polymerization and precipitation of heme and non-heme iron, respectively. In particular, peptides rich in the amino acid cysteine, have been implicated in absorption improvement by serving as a ligand for iron. This binding and subsequent reduction to ferrous iron prevent the formation of insoluble complexes that reduce non-heme iron absorption (Taylor et al. 1986). Only sulfur-containing amino acids like cysteine are known to exhibit this meat factor effect, but cysteine’s low stability at neutral pH has led researchers to believe that it is cysteine-containing peptides (and not cysteine alone) that produce the effect (Layrisse et al. 1984; Taylor et al. 1986).

In all, there is sufficient evidence reporting on the “meat factor” and its positive effect on non-heme iron absorption. However, the mechanisms by which animal tissues enhance iron availability remain unknown. Furthermore, there is still uncertainty as to what specific
components participate in this reaction and whether these components function alone or in combination with other dietary factors.

In summary, the total amount of iron assimilated from the diet is influenced by many key features (i.e. form of iron, presence of dietary iron facilitators and inhibitors, and individual health and iron status), each of which needs to be assessed when evaluating dietary iron absorption.

Methods for Assessing Iron Bioavailability

The bioavailability of a nutrient can be subdivided into three phases: 1) availability in the intestinal lumen for absorption, 2) absorption into the bloodstream and finally 3) utilization by the body (Fairweather-Tait, 1993). As this list confirms, bioavailability is an intertwining of various physiological and dietary processes that make quantification of bioavailability difficult. However, various techniques have been established and utilized by researchers both in vitro and in vivo.

Chemical balance technique

The chemical balance technique is an earlier method used to study iron bioavailability. This method calculates iron absorption as the difference between total iron ingested from the diet and the amount excreted in the feces. Because iron is not actively excreted from the body, the retention of iron is assumed to represent “apparent” iron absorption (Wienk et al., 1999). The use of the chemical balance method has decreased over time, as it is time-consuming, sensitive to calculation error, and costly. This makes studies difficult to conduct on a large-scale basis. This method also gives no information about iron absorption in different meals.
For these reasons, chemical balance methods are uncommon in present day research.

**Radioisotope Labeling**

The introduction of radioisotopes proved a powerful tool for studying nutrient and mineral bioavailability. This method allows researchers to label specific foods, either extrinsically or intrinsically, with radio iron and thus quickly measure non-heme iron absorption from single meals. Studies with radio-labeled foods established the idea that absorption differs between different foods.

Extrinsic labeling involves the addition of a trace amount of radiolabeled iron (usually 59FeCl3 or 59FeSO4) to a test diet. Bioavailability is determined by multiplying the percent of absorbed radioiron by the percent of non-heme iron from the meal/test diet and extrapolating the amount of radioiron absorbed to the quantity of iron given (Hallberg et al. 1983). A major advantage to this method is that it can be used to identify and isolate the factors that affect non-heme iron absorption from composite meals. However, it is important to be aware of both heme and non-heme iron content when using this method even if the experiment only tags non-heme iron (Bjorn-Rasmussen and Hallberg 1974). This method is based on the principle of a complete isotopic exchange between the extrinsic tag and the iron in the diet. The extrinsic labeling method has proven valid for various foods (Bjorn-Rasmussen et al., 1973) based on the complete exchange between endogenous non-heme iron and the extrinsic tags. However, incomplete exchanges have been shown to occur in some foods (Bjorn-Rasmussen et al., 1973) and thus this method cannot be validated for all foods. Isotopic exchange is dependent on various mechanical and chemical factors including rate of
diffusion, degree of mixing, and binding and dissociation from other iron inhibitors (Bjorn-Rasmussen et al., 1973). Therefore, extrinsic labeling is not appropriate for application in foods that contain large amounts of insoluble iron for risk of inaccuracy.

In comparison to extrinsic labeling, intrinsic labeling is the less popular of the two techniques (particularly in regards to applications in humans). Intrinsic radio-iron labeling is a method by which an iron isotope is naturally integrated into a food. Intrinsic tagging allows researchers to evaluate and isolate the effect of dietary inhibitors and enhancers on iron absorption from specific foods. Unfortunately, this method does not permit total determination of iron absorption from the entire diet because it labels individual foods (and not an entire meal). In addition intrinsic labeling can be expensive, labor intensive, and time consuming.

**Animal Models**

Many researchers have utilized animal models, particularly rodent models, to estimate iron bioavailability. However, research comparing iron bioavailability in rats and humans has shown considerable discrepancy (Reddy and Cook, 1991). In one study, identical methods (test meals) were used to compare the change in iron absorption in rats and humans when supplemented with dietary enhancers and inhibitors. The rats showed no change in absorption when exposed to any other dietary component. According to the authors, this suggests that non-heme iron absorption data in rats cannot be extrapolated to humans (Reddy and Cook 1991).

A major limitation of using these types of models is the physiological differences between humans and rodents. These differences make it difficult to accurately extrapolate
data to a human population. In relation to iron bioavailability, rodents utilize different mechanisms compared to humans. For example, heme and non-heme iron (absorbed separately in the human body) are thought to share similar pathways in rodent guts. Another disparity between human and rat physiology is the innate presence of inhibitors and enhancer components in rats. Rodents possess the ability to synthesize Vit C, a dietary enhancer of non-heme iron (humans cannot synthesize Vit C and must obtain it from their diet.) Endogenous vitamin C could substantially alter non-heme iron absorption. Rat tissue also contains the enzyme phytase, which catalyzes the breakdown of phytate a known inhibitor of iron absorption. Rats and other rodents also practice coprophagy (the consumption of their feces), which recycles nutrients and can confound results. Pig models have also been suggested in nutrition studies due to the similarities of their intestinal physiology and digestion with humans. However, cost of care limits their application and use of the porcine model remains uncommon (Patterson et al., 2008).

In vitro digestion/ Caco-2 cell model

Since its establishment by Raymond Glahn in 1996, the Caco-2 cell digestion model has accelerated scientific knowledge on iron bioavailability. It was a unique technique in that it was an in vitro laboratory model that coupled simulated food digestion with a human intestinal cell line (Caco-2). The model has had broad applications for studying the relative bioavailability of staple foods (Boato et al. 2002; Huh 2004; Glahn et al 2008), dietary supplements (Failla et al. 2004), and baby formulas and food (Glahn et al. 1998; Glahn et al. 2004; Viadel et al. 2007; Cilia et al. 2008). Caco-2 cell/digestion models are preferred over other bioavailability assessment tools because they allow for bigger sample sizes to be tested
in a rapid manner and also they allow the researcher to focus on the effect of dietary factors alone (instead of in combination with physiological factors).

As the name implies, this *in vitro* digestion/Caco-2 cell model involves the simulation of gastrointestinal digestion of food; the digested food is then combined with Caco-2 cells to mimic intestinal absorption. The bioaccessible iron can then enter the Caco-cells (thus becoming bioavailable iron). The amount of iron that has entered the cell is then quantified in the form of ferritin using commercial assay kits. Caco-2 cell ferritin formation serves as the biomarker for iron uptake in this method (Glahn et al. 1998) and has been qualitatively validated (Miller & Glahn 1998). Glahn et al. (1998) demonstrated that iron that was readily available induced a greater ferritin formation in Caco-2 cells than less available forms. When Caco-2 cell monolayers were exposed to foods with highly bioavailable iron (i.e. meat and fish) there was an increase in relative ferritin formation versus foods low in bioavailable iron (i.e. corn and beans). From their results, the researchers concluded that Caco-2 cell ferritin formation occurs in direct proportion to the amount of iron readily available to cells. Thus, Caco-2 cell ferritin formation can be utilized as a valid biomarker for iron uptake, providing an accurate estimate of iron bioavailability (Glahn et al. 1998).

Because ferritin formation occurs naturally in response to iron uptake, its use as a biomarker eliminates issues experienced using radiolabeled iron which has the potential to nonspecifically bind to the cell surface. If the amount of non-specifically bound iron is not accounted for, values obtained from such measurements are likely to be inaccurate of actual iron uptake (Glahn et al. 1998). However, the use of ferritin as an indirect measure of iron bioavailability does have its limitations. Factors that affect ferritin formation, independent of iron uptake, can limit the usefulness of this biomarker. For example any substances that
reduce ferritin synthesis may mistakenly be thought to be a cause of iron inhibition. Also the simulated in vitro digestion model established by Glahn, requires that the Caco-2 cells be cultured on 6-well plates. This does not account for movement of iron or any activity that occurs across the basolateral end of the cell as the cells are firmly attached to the base of the plate.

Despite these issues, the use of Caco-2 cell ferritin formation as a biomarker has been successfully used as a valid method for indirectly measuring iron absorption in many studies. These studies have demonstrated that ferritin formation is qualitatively more accurate than radiolabeled iron. Estimations of iron bioavailability obtained from this in vitro model have been well correlated with those obtained in human studies. In addition, utilization of Caco-2 cells is also cost and labor effective, and avoids the use of radioactive iron with ferritin formation instead acting as a biomarker for iron absorption.

**Current Programs and Agencies that Combat Iron Deficiency**

In light of the magnitude of the problem, global goals and interventions have been established in conjunction with substantial funding aimed toward alleviating iron malnutrition (WHO 2001). There are three main strategies used, either alone or in combination, to combat iron deficiency: iron supplementation, iron fortification in foods, and dietary modification to improve iron intake and bioavailability (Heath et al., 2002; Hoppe et al., 2008; Polin et al., 2013). Many of these programs are currently implemented around the world, but unchanging global frequencies of deficiency serve as evidence that the effectiveness of such intervention strategies as a whole is unsuccessful. Iron supplementation of ferrous iron salts (e.g. ferrous sulphate) is a cost effective strategy that can be targeted specifically toward high-risk groups.
such as pregnant women and infants but proper distribution and compliance issues are major limitations (Baltussen et al., 2004; Polin et al., 2013). In addition, supplemental iron is often poorly accepted within the human gastrointestinal tract (Zimmermann and Hurrell, 2007).

Iron fortification is a sustainable, cost-effective long-term option to increasing iron intake in large populations. However, less soluble and less bioavailable forms of iron are currently added to foods such as flour, milk and cereal to avoid undesirable sensory changes in foods (color changes, off flavors, etc.) (Zimmermann and Hurrell, 2007). The limitations of iron supplementation and food fortification place importance on dietary modification. This food-based approach focuses on dietary diversification and/or modification to help improve availability, access to, and utilization of foods that promote increased iron intake and absorption.

In summary, iron deficiency remains a public health problem despite its documented negative impact on health. While dietary modification is the most tenable approach to combating iron deficiency, it is often the most complex as changes to dietary practices and preferences are subjective and difficult. In addition, foods that provide the most bioavailable forms of iron, such as meat, are expensive or limited. Although food fortification and iron supplementation have shown improvement, the associated side effects (low palatability, compliance, and high costs) are difficult to overcome. Thus, further funding and research is required to implement sustainable and economically feasible, food-based strategies to ameliorate this global epidemic.
iv. SEAWEED AS A POTENTIAL SOURCE OF BIOAVAILABLE IRON

Seaweed Classification

Seaweed is a colloquial term that lacks in formal definition, but encompasses macroscopic, multi-cellular, benthic marine algae. These macroalgae may be classified into one of three groups based on pigmentation: red algae (Rhodophyta), green algae (Chlorophyta), or brown algae (Phaeophyta). In addition, some blue-green algal species (Cyanobacteria) are sometimes considered to be seaweeds. These marine algae are found throughout the world, as they are able to grow in water temperatures ranging from cold, to temperate, to tropical. As a result of their extensive distribution, edible seaweeds represent a durable, virtually inexhaustible natural resource, existing in large quantities all along the Pacific and Atlantic coasts. Nevertheless, despite their abundance and potential nutritional, pharmaceutical and agricultural applications, seaweeds have been poorly exploited (Dhargalkar and Pereira, 2005).

There are more than 145 identified edible seaweed species worldwide (Zemke-White & Ohno, 1999). Most of these seaweeds are utilized primarily for direct consumption as a food product. Seaweeds are traditionally consumed in highest abundance in Asian and Pacific diets, particularly in Japan, Korea, and China. Different reports have estimated consumption in Asia between 4-7g/ day of dried seaweed and 12 g/d of both dry and wet weight (Nisizawa et al., 1987; Nagataki, 2008; Zava and Zava, 2011). Although seaweeds have experienced a recent general acceptance in the diets of Western countries, consumption estimates remain minimal with lower rates at <1g/d (Nisizawa et al., 1987; Nagataki, 2008; Zava and Zava, 2011). Nori, wakame, and kombu represent the most commonly eaten seaweeds, constituting over 95% of global seaweed consumption (McHugh, 2003).
In addition to direct consumption, seaweeds are utilized industrially as a source of hydrocolloids, e.g. agar and carrageen (brown algae), and alginates (red algae) for food and pharmaceutical additives. Seaweeds are also used to a lesser extent as an ingredient in animal feeds and fertilizers because of their purported high nutritive value.

Nutritional Content in Seaweeds

Seaweeds are considered sources of many nutrients including dietary fiber, vitamins, minerals, protein and bioactive compounds. Due to their nutritive value, seaweeds have been traditionally used as a food worldwide (McHugh, 2003). These dietary components may help reduce the risk of heart disease, cancer, and other chronic diseases. Therefore there is interest in the use of edible seaweeds in the development of low-cost, highly nutritive diets for human and animal nutrition.

Edible seaweeds are rich in non-starch polysaccharides that cannot be entirely digested by human digestive enzymes and thus act as a source of dietary fiber. The literature has reported diverse ranges of total dietary fiber content ranging from 31%-62% dry matter (Sanches-Machado et al., 2004; Mabeu and Fleurence, 1993). The fiber content, particularly soluble fiber, in some seaweed varieties is higher than those found in most fruits and vegetables (Goni & Saura-Calixto, 2002). Consequently, seaweed consumption in relation to dietary fiber has shown the following health benefits: (1) a lowered glycemic response when consumed with foods that have a high glycemic load, (2) increase in fecal bulk, and (3) promotion of the growth of beneficial intestinal microflora (Goni and Urbano, 2002). In a study done by Goni & Urbano (2002), rats fed seaweed-containing (Nori and Wakame) diets
for 2 weeks showed a significant increase in stool weight compared to those who consumed a control diet. This difference was attributed to the increase in dietary fiber intake.

Some types of seaweed provide significant amounts of water-soluble vitamins, including Vitamin C and the B-vitamins (MacArtain et al., 2007). Vitamin C participates in many vital reactions in the human body, including the absorption of various iron forms and facilitating iron transport across the microvillus membrane. In previous studies, some green and brown seaweed showed high levels of vitamin C ranging from 500-3000 mg/kg dry matter (McDermid and Stuercke, 2003).

Furthermore, some seaweeds have been shown to provide significant amounts of protein and other nitrogenous compounds (free amino acids, nitrate, nucleic acids, etc.) (MacArtain 2007). Protein values between seaweeds are difficult to compare due to the use of different values of the nitrogen-to-protein conversion factor. However, most studies utilize a conversion factor of 6.25 and have shown protein contents ranging from 5-35% dry matter (Mabeau and Fleurence, 1993; Burtin 2003; Dawczynski et a., 2007). Similar to plant proteins, seaweeds generally contain all of the essential amino acids with tryptophan (Dawczynski et al., 2007) and methionine (Ramos et al., 2000) being the main limiting amino acids.

In addition, some seaweeds also provide fat-soluble vitamin E and carotenoids (precursor forms of Vitamin A) (McDermid 2003; Ortiz et al., 2006; Burtin, 2003). Beta-carotene and beta-carotene rich foods have been associated with the enhancement of iron bioaccessibility (Gracia-casal et al., 1998; Gautam et al., 2010). This compound is believed to form a soluble complex with iron, thus preventing the inhibitory effect of phytate and polyphenols.
Polyphenols also occur in various amounts in plant foods and have been shown to occur at high levels in seaweeds (McDermid and Stuercke, 2003). It may be that seaweed polyphenols are formed as a defense mechanism against herbivores and to reinforce seaweed tissue against heavy wave exposure. However polyphenols have been shown to form insoluble complexes with iron, thus decreasing its uptake by the small intestine.

The total lipid content of algae is very small, constituting between 1-5% of dry matter (MacArtain et al., 2007). However, a majority of the lipid content is attributed to long-chain n-3 and n-6 polyunsaturated fatty acids, namely eicosapentaenoic acid (EPA, n-3, C20:5) and arachidonic acid (AA, n-6, C20:4) (Jahreis et al., 2006) which may reduce the risk of cardiovascular disease and diabetes (Maeda et al., 2008). In most algae, palmitic acid is the most abundant saturated fatty acid (Ortiz et al., 2006; Kumari et al., 2010). Consumption of seaweed could increase the dietary supply of n-3 fatty acids, and serve as an alternative source of PUFAs to fish and fish oils.

Taken together, seaweeds are considered to be significant sources of dietary fiber, vitamins, minerals (Burtin, 2003; Bocanegra et al. 2003), and protein with low lipid levels. Thus seaweeds could represent a low calorie, nutrient dense food product. However, nutritional content is highly variable between species and caution must be taken when generalizing nutritional data to species.

**Phaeophyta**

The phaeophyta (brown algae) are so classified based on the presence of brown carotenoid pigments in their cell chloroplasts including fucoxanthin and the photosynthetic pigments chlorophylls \(a\) and \(c\). With only a few exceptions, most phaeophyta grow in colder
waters, many in the tidal zone, where they are subjected to great stress from wave action; others may grow in deep water. Among the brown algae are the largest of all algae, the giant kelps, which may reach lengths of over 100 ft.

Brown algae typically have low fat and protein content. In phaeophyta, the protein content is commonly low, between 5-15% of dry matter (Burtin 2003; Dawczynski et al., 2007); relatively low (Ruperez and Saura-Calixto 2001) compared to red algae species (Fleurence 1999; Ruperez and Saura-Calixto 2001). Phaeophyta are also known to contain a wide variety of bioactive compounds. In a study by Kelman et al (2012) phaeophyta collected in Hawaii showed the highest antioxidant activity versus other red and green algae tested. In addition, studies have shown consistently higher levels of the heavy metals iodine and arsenic in brown algae compared to rhodophyta and chlorophyta (Teas et al., 2004; Dawczynski et al. 2007).

A. Hijiki (Hizikia fusiforme)

*Hizikia fusiforme* (Hijiki) is a brown seaweed characterized by dark pigmentation and can either be collected from the wild or cultivated. Excessive amounts of the pigment phlorotannin, give the seaweed an astringent, bitter taste and thus it is often subjected to heavy amounts of cooking and processing before consumption. Typically Hijiki is sold dry to be reconstituted by soaking or washing before consumption in various stir-fry or soup recipes (Hanaoka et al., 2001).

Like most brown seaweeds, the fat content in Hijiki is relatively low (~1.5%); 20-25% of which is attributed to the essential fatty acid eicosapentaenoic acid (EPA) (Nisizawa et al., 1987).
Particularly hijiki over other seaweeds has shown high levels of inorganic arsenic. Arsenic in its inorganic form has been associated with serious health concerns and as a result the FDA has set the tolerable daily intake at 130 µg. The toxic effects of chronic hijiki consumption are unclear, but ingestion by consumers at a single meal is estimated between 5-10 g (dry weight) (Hanaoka et al., 2001). Of this amount, Ichikawa et al (2006) demonstrated that about 90% of the total arsenic in Hijiki can be removed by the cooking process. Thus it has been suggested that the significant decrease in arsenic concentration brought about by the washing/soaking of hijiki is, at least, part of the reason why people are not poisoned by excessive ingestion of hijiki (Hanaoka et al., 2001; Ichikawa et al., 2006).
Table 1.4 - Macro and Trace Mineral Content per gram dry weight in selected species of phaeophyta.

<table>
<thead>
<tr>
<th>Species</th>
<th>Geographic Origin</th>
<th>Reference</th>
<th>Ca (mg/g DW)</th>
<th>K (mg/g DW)</th>
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Species Geographic Origin Reference Ca (mg/g DW) K (mg/g DW) Mg (mg/g DW) Na (mg/g DW) Al (µg/g DW) As (µg/g DW) Cd (µg/g DW) Cu (µg/g DW) Fe (µg/g DW) Hg (µg/g DW) I (µg/g DW) Se (µg/g DW) Zn (µg/g DW)
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<td>139.1 ± 95.66</td>
<td>70.6 ± 87.8</td>
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B. Wakame (*Undaria pinnatifida*)

*Undaria pinnatifida* (Wakame) has been a widely consumed edible seaweed in Korea, China and Japan for hundreds of years (McHugh, 2003). This sea vegetable is otherwise known as “*miyeok*” in Korea, “sea mustard” in English, “*wakame*” in Japan, and “*quandaicai*” in China. Recently, other Western and European countries such as the United States, United Kingdom, France and Spain have introduced this seaweed into their diet (Torres et al., 2004). Wakame occurs on rocky shores and bays in the temperate zones of Japan, Korea and China, growing best on rocks and reefs at temperatures between 5° and 15°C (Chapman and Chapman, 1980). Wakame contains calcium, potassium, iodine, and niacin and is a rich source of the omega-3 fatty acid, EPA (Dawczynski et al., 2007) and soluble dietary fiber (Ruperez and Saura-Calixto, 2001).

Although wild harvesting still occurs, most packaged wakame originates from cultivated plants. Wakame is typically used for making broth (i.e. soaked in boiling water then removed), or in seaweed salads (Chapman and Chapman, 1980).

C. Kombu (*Laminaria spp.*)

Kombu (kelp in English, “*dashima*” in Korean, “*Konbu*” in Japanese, or “*haidai*” in Chinese) is an edible brown seaweed derived from a mixture of *Laminaria* species. These sea vegetables grow best on rocks and reefs in calmer tidal zones, between 3° and 20°C (Chapman and Chapman, 1980) and are cultivated on long ropes in Japan and China.

*Laminaria* species are sources of iron, dietary fiber and iodine. The concentration of iodine in *Laminaria* species is consistently high ranging from 2.0-4.25 mg/g dry weight (Hou and Yan, 1998; Teas et al., 2004; MacArtain, 2007). Kombu has also been found to
accumulate high levels of arsenic and aluminum from its marine environment (Hou and Yan, 1998; Besada et al., 2009; Rodenas et al., 2009; Van Netten et al., 2000).

**Rhodophyta (red algae)**

There exist about 8,000 species of red algae, most of which are marine (Chapman and Chapman, 1980). They are found in the intertidal and subtidal zones in temperate, tropical, and cold-water environments. The red color of these algae is attributed to the dominance of the phycobilin pigments, phycoerythrin and phycocyanin, which reflect red light and absorb blue light. Rhodophyta can range in color from orange to purple and this color variation is a result of the presence of other pigments such as chlorophyll, lutein, and beta-carotene that may give the seaweed a green or bluish tinge. Their ability to absorb blue light (which penetrates water at a greater depth than other wavelengths) allows rhodophyta to thrive in deeper waters versus the phaeophyta and chlorophyta.

Several red algae are of economic value, either as producers of secondary products (Gracilaria) used in the food and pharmaceutical industries or as a direct food (Nori, Dulse etc). Nutritionally, rhodophyta are recognized as a source of essential amino acids (Dawczynski et al., 2007), as well as having high levels of soluble protein (Fleurence et al; 2012).

A. Nori (*Porphyra* spp.)

Of the edible rhodophyta, *Porphyra* (laver in English, nori in Japanese, or “*gim*” in Korean) is the most well-known genus. Edible nori derives from either the *Porphyra tenera* or *Porphyra yezoensis* species and is most commonly eaten dried or roasted as a wrapping,
seasoning, or condiment (McHugh, 2003). Processing of wet Porphyra into dried sheets of nori varies between regions and involves extensive rinsing, chopping and drying and/or heating. Valued at over $1 billion, nori is the single most valuable marine crop (McHugh, 2003).

Nori is a source of protein, fiber (Ruperez & Saura-Calixto, 2001), omega-3 fatty acids (Dawczynski et al., 2007) and vitamin C.

B. Ogos (Gracilaria spp.)

There are many edible seaweed species belonging to the genus Gracilaria, commonly known as sea moss in English or “ogo” in Japanese. Some of the most common and easily harvested ogos grow in shallow waters on reefs flats or in lagoons (Novaczek 2001). These seaweeds are a purported source of vitamins C, A, and B2, potassium, and the minerals calcium, and iron. Previous studies have also identified Gracilaria species as a source of bioactive compounds (Kelman et al., 2012).

In Western culture, Gracilaria are used as sources of agar but fresh Gracilaria has been collected and sold as a sea vegetable in Hawai‘i for several decades (McDermid and Stuercke, 2003). The mixture of ethnic groups in Hawai‘i (e.g. Hawaiians, Filipinos, Koreans, Japanese, Chinese etc.) creates an unusual demand and the stocks available from natural sources have, at times, limited supply (McDermid and Stuercke, 2003). Now ogo is being successfully cultivated in Hawai‘i, producing up to 6 tons fresh weight per week (Department of Land and Natural Resources 2002). Gracilaria can be eaten either raw or cooked, but it is commonly added fresh to raw, salted fish (limu poke in Hawaiian).
*G. salicornia*, a species of *Gracilaria* common in warm waters, has been a recent source of concern for the marine life in Hawai‘i. Also known as Gorilla ogo, this seaweed has spread over 5 kilometers from its point of introduction on O‘ahu since it was introduced in 1978 (Smith et al., 2004). This alien species is thought to compete with the native reef algae, such as *G. coronopifolia*, for substrate on the reef flat. When other more desirable cultured *Gracilaria* species or the wild *G. coronopifolia* are not available for consumption, *G. salicornia* is used as a substitute. Its "crunchiness" is gaining favor, and this species has been sold under the name "ogo" in Oahu, HI (Smith 2004).

C. Dulse (*Palmaria palmata*)

Dulse, a red algae with leathery fronds is harvested mainly from mid-May to mid-October in Ireland and the shores of eastern Canada and Maine. After picking, the seaweed is laid out to sun dry for 6-8 hours upon which it is either broken into flakes or ground into powder for use as a seasoning (Chapman and Chapman, 1980).

Dulse is a source of the minerals iron and potassium. This red alga has also shown considerable levels of polyphenolic compounds. The antioxidant capacity of dulse may be attributed to its location in the intertidal zone where it is exposed to stressors from UV light and fluctuations in water levels. Thus, these plants would require protection against cell damage (Yuan et al., 2005).
### Table 1.5- Macro and Trace Mineral Content per gram dry weight in selected species of rhodophyta

<table>
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<th>Species</th>
<th>Geographical Origin</th>
<th>Reference</th>
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<th>K (mg/g DW)</th>
<th>Mg (mg/g DW)</th>
<th>Na (mg/g DW)</th>
<th>Al (µg/g DW)</th>
<th>As (µg/g DW)</th>
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<td>Porphyra sp.</td>
<td>Korea</td>
<td>Dawczynski et al., 2007</td>
<td>3.39 ± 0.92</td>
<td>27.2 ± 11.4</td>
<td>3.5 ± 0.5</td>
<td>5.87 ± 3.31</td>
<td>4.25 ± 0.13</td>
<td>2.88 ± 0.13</td>
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<tr>
<td></td>
<td>Species</td>
<td>Geographic Origin</td>
<td>Reference</td>
<td>Ca (mg/g DW)</td>
<td>K (mg/g DW)</td>
<td>Mg (mg/g DW)</td>
<td>Na (mg/g DW)</td>
<td>Al (µg/g DW)</td>
<td>Cd (µg/g DW)</td>
<td>Cu (µg/g DW)</td>
<td>Fe (µg/g DW)</td>
<td>Hg (µg/g DW)</td>
<td>I (µg/g DW)</td>
<td>Se (µg/g DW)</td>
<td>Zn (µg/g DW)</td>
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</tr>
<tr>
<td>1</td>
<td><em>Porphyra</em> sp.</td>
<td>China</td>
<td>Dawczynski et al., 2007</td>
<td>3.11 ± 0.73</td>
<td>29.0 ± 3.1</td>
<td>2.3 ± 0.09</td>
<td>4.0 ± 0.1</td>
<td>8.77 ± 1.36</td>
<td>0.46 ± 0.06</td>
<td></td>
<td>0.536 ± 0.022</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>2</td>
<td><em>Porphyra</em> sp.</td>
<td>Qingdao, China</td>
<td>Hou and Yan, 1998</td>
<td>6.28 ± 0.19</td>
<td>3.07 ± 0.07</td>
<td></td>
<td>3.18 ± 0.08</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.375 ± 0.024</td>
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<td></td>
</tr>
<tr>
<td>3</td>
<td><em>Porphyra</em> sp.</td>
<td>Galicia, Spain</td>
<td>Moreida-Pineiro et al., 2007</td>
<td>2.2 ± 0.1</td>
<td>13 ± 2</td>
<td>3.48 ± 0.32</td>
<td>7.06 ± 3.11</td>
<td>9.70 ± 1.20</td>
<td>3.18 ± 0.08</td>
<td></td>
<td></td>
<td>0.438 ± 0.032</td>
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</tr>
<tr>
<td>4</td>
<td><em>Porphyra</em> sp.</td>
<td>Margarita Island, Venezuela</td>
<td>Garcia-Casal et al., 2007</td>
<td>115.7-140.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>5</td>
<td><em>Porphyra tenera</em></td>
<td>Japan</td>
<td>Van netten et al., 2000</td>
<td>4</td>
<td>29</td>
<td>0.27</td>
<td>6.3</td>
<td>110</td>
<td>0.44</td>
<td>17</td>
<td>2</td>
<td>31</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>6</td>
<td><em>Porphyra tenera</em></td>
<td>Galicia, Spain</td>
<td>Ruperez, 2002</td>
<td>3.90 ± 0.17</td>
<td>35 ± 0.71</td>
<td>5.65 ± 0.11</td>
<td>36.2 ± 1.15</td>
<td>2.6</td>
<td>29</td>
<td>0.83</td>
<td>8.9</td>
<td>190</td>
<td>0.24</td>
<td>185</td>
<td>1.53</td>
</tr>
<tr>
<td>7</td>
<td><em>Porphyra</em> vietnensis</td>
<td>Big Island, HI</td>
<td>McDermid &amp; Stuercke, 2003</td>
<td>2.9</td>
<td>39.7</td>
<td>7.8</td>
<td>7</td>
<td>7</td>
<td>154</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td><em>Porphyra</em> vietnensis</td>
<td>India</td>
<td>Rao and Ganesan, 2007</td>
<td>3.35 ± 0.84</td>
<td>2.49 ± 0.64</td>
<td>4.95 ± 1.01</td>
<td>52.3 ± 3.2</td>
<td>16.0 ± 1.8</td>
<td>2.9 ± 0.1</td>
<td>8.3 ± 0.2</td>
<td>1370 ± 9.9</td>
<td>0.2 ± 0</td>
<td></td>
<td></td>
<td>1.95 ± 0.01</td>
</tr>
<tr>
<td>9</td>
<td><em>Porphyra</em> vietnensis</td>
<td>India</td>
<td>Dawczynski et al., 2007</td>
<td>3.11 ± 0.73</td>
<td>29.0 ± 3.1</td>
<td>2.3 ± 0.09</td>
<td>4.0 ± 0.1</td>
<td>8.77 ± 1.36</td>
<td>0.46 ± 0.06</td>
<td></td>
<td></td>
<td>0.536 ± 0.022</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>
Table 1.6- Macro and Trace Mineral Content per gram dry weight in selected species of chlorophyta

<table>
<thead>
<tr>
<th></th>
<th>Species</th>
<th>Geographical Origin</th>
<th>Reference</th>
<th>Ca (mg/g DW)</th>
<th>K (mg/g DW)</th>
<th>Mg (mg/g DW)</th>
<th>Na (mg/g DW)</th>
<th>Al (µg/g DW)</th>
<th>As (µg/g DW)</th>
<th>Cd (µg/g DW)</th>
<th>Cu (µg/g DW)</th>
<th>Fe (µg/g DW)</th>
<th>Hg (µg/g DW)</th>
<th>I (µg/g DW)</th>
<th>Se (µg/g DW)</th>
<th>Zn (µg/g DW)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Ulva lactuca</td>
<td>Weihai, China</td>
<td>Hou and Yan, 1998</td>
<td>4.9</td>
<td>50.4</td>
<td>32</td>
<td>57.3</td>
<td>3077</td>
<td>4.09</td>
<td>2034</td>
<td>53.8</td>
<td>&lt;0.15</td>
<td>16</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Ulva lactuca</td>
<td>Karachi, Pakistan</td>
<td>Rizvi and Shameel, 2001</td>
<td>8.54±5</td>
<td>32.5±5</td>
<td>36.9</td>
<td>2.3</td>
<td>7.12±5</td>
<td>382.5±5</td>
<td>0.354±0.002</td>
<td>19.1±1.6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Ulva spp.</td>
<td>Margarita Island, Venezuela</td>
<td>Garcia-Casal et al., 2007</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>210.0-2366</td>
<td></td>
<td></td>
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<td></td>
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</tr>
</tbody>
</table>
Chlorophyta

Chlorophyta, or green algae, are the most diverse division of seaweeds with over 7,000 identified species (Hoek et al., 1995). This group occupies a wide array of habitats including terrestrial, freshwater and marine environments usually in shallow water and often attached to rocks or other substrate. Similar to land plants, the dominant pigments in chlorophyta are chlorophylls a and b, which give green algae its distinct color (Chapman and Chapman, 1980).

The green algae are characterized by protein concentrations ranging from 6-12% of dry matter and generally have higher levels of vitamin C compared to red and brown algae, ranging between 220-300 mg/ 100 g dry matter (Burtin, 2003; McDermid and Stuercke, 2003).

A. Sea lettuce (Ulva lactuca)

*Ulva lactuca*, or sea lettuce, is a brightly colored, ruffled seaweed that belongs to the chlorophyta. Sea lettuce propagates best in shallow, cooler waters attached to a hard substrate. Although grainy in texture, *Ulva* can be finely chopped and added raw to salads or other dishes. This seaweed can also be made into various soups, sauces, relishes, or seasonings (Novaczek 2001).

Nutritionally, sea lettuce is a source of iodine, aluminum, iron, and contains variable amounts of vitamins A, B and C (Nisizawa et al., 1983; Hou and Yan, 1998). Like most seaweeds, *Ulva* species also provide considerable amounts of protein and essential amino acids, dietary fiber and polyunsaturated fatty acids (Ortiz et al., 2006).
B. Wawae’iole (*Codium edule*)

*Codium edule* (“wawae’iole” in Hawaiian) is a dense, tubular, green seaweed that grows attached to rocks and reefs in areas of strong tidal currents. Wawae’iole is eaten either raw, pickled or cooked in stews and soups and is a source of vitamin A (β-carotene is thought to be the principal source) (Novaczek 2001). *Codium* species are characterized by high protein contents (13.7–10.8% of dry weight), and a low lipid content (0.7–15.0% of dry weight) (Ortiz et al., 2009).

**Industrial Value and Uses**

Seaweed is a versatile product, and its uses are vast (Table 1.7) (Chapman and Chapman, 1980). Seaweed products significantly contribute to the economies of island nations such as Japan and others with large coastal areas.

**Table 1.7- Commercial uses of seaweeds**

<table>
<thead>
<tr>
<th>USES</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Food</td>
<td>Used for human consumption in the preparation of salads, soups, seasonings, and vinegars.</td>
</tr>
<tr>
<td>Industrial raw material</td>
<td>i.e., Agar, carrageenan, and algin</td>
</tr>
<tr>
<td>Animal Feed</td>
<td>High nutritive value seaweeds are mixed in with animal fodder to provide nutrients.</td>
</tr>
<tr>
<td>Manure</td>
<td>Bioaccumulation capacity of nutrients from the soil provides rich sources of vitamins and minerals.</td>
</tr>
<tr>
<td>Biological waste treatment</td>
<td>Bioaccumulation capacity of metals from aquatic mediums</td>
</tr>
</tbody>
</table>

The seaweed industry has an estimated total annual value of $6 billion, with food products alone constituting $5 billion of this (FAO 2006). The algal industrial products of current greatest economic importance are the extracted hydrocolloids: agar, carrageenan, furcellaran and algin. These hydrocolloids have gained commercial significance in Western countries as a food ingredient because of their water retaining and gelling
properties. Other, less popular uses include fertilizer and animal feed additives (McHugh, 2003).

Sources of seaweed include both wild and cultivated crops. Seaweed farming has seen a drastic increase in the past decade as demand has exceeded natural supply (FAO 2006).

**Seaweeds in Hawai‘i**

There are an estimated 500 species of algae in Hawai‘i and the Pacific alone (McDermid 2003; Abbott and Huisman, 2004). In Hawai‘i, before contact with Western settlers, seaweed or *limu* was a staple food and accompanied most meals. *Limu* was traditionally treated as a vegetable by the native Hawaiians, mixed fresh with cooked meats, fish, shellfish or taro (Novaczek 2001). Unfortunately of the 70 edible marine seaweeds classified in the beginning of the century, only 29 can be identified today by both their Hawaiian and common names (Abott 1999). The commercial harvest of Hawaiian *limu* has risen over the years as daily consumption has increased. Hawaiian seaweed cultivation was valued at $38,849 in 2002 (Department of Land and Natural Resources 2002) and today is utilized by a variety of ethnic backgrounds. At present, aquacultured *Gracilaria* species (*G. coronopifolia, G. parvispora, G. tikvahiae*) and wawai’eiole are the seaweeds most often sold and consumed (McDermid and Stuercke, 2003) in markets in Hawai‘i.
Mineral Content of Seaweeds

Seaweeds concentrate minerals from the seawater from which they are grown, and consequently are high in many macro and trace elements. Minerals such as iron and copper are present in seaweeds at higher levels than in many well-known terrestrial sources of minerals, such as meats and spinach. For example, there is more iron in an 8 g serving of dry Palmaria palmata (dulse) than in 100 g of raw sirloin steak (6.4 mg versus 1.6 mg, respectively) (MacArtain, 2007).

Few studies have reported on the mineral and vitamin content and bioavailability of Hawaiian seaweed species. Several of the algal species propagated in Hawai‘i are related to species found in other parts of the globe but ecological variation makes it difficult to generalize nutritional data to one genus. McDermid reported values comparable to those found in similar species from other parts of the world (McDermid and Stuercke, 2003). Ash values for Hawaiian Gracilaria species were higher than the published values for Gracilaria from other parts of the world (Burkholder et al., 1971; Norziah & Ching, 2000). In addition, concentrations of most major essential elements in Hawaiian seaweeds were similar to those reported for the same species in other studies (Robledo & Freile Pelegrin, 1997).

Algal mineral content is generally higher than that of land plants and animal products (Ito & Hori, 1989; Ortega-Calvo et al., 1993) but varies according to species, processing, and environmental conditions (Table 1.8).
Table 1.8- Factors that affect mineral composition in seaweeds

<table>
<thead>
<tr>
<th>Environmental factors</th>
<th>Literature Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water temperature</td>
<td>Cho et al., 1995; Fleurence 1999</td>
</tr>
<tr>
<td>Light intensity</td>
<td>Cho et al., 1995; Fleurence 1999</td>
</tr>
<tr>
<td>Geographical origin</td>
<td>Cho et al., 1995; Kupper et al., 1998; Muniz et al., 2008</td>
</tr>
<tr>
<td>Wave exposure</td>
<td>Teas et al., 2004</td>
</tr>
<tr>
<td>Oceanic residence time</td>
<td>Gall et al., 2004</td>
</tr>
<tr>
<td>Period in life cycle</td>
<td>Gall et al., 2004</td>
</tr>
<tr>
<td>Pollution/presence of other minerals</td>
<td>Kupper et al., 1998; Muniz et al., 2008</td>
</tr>
<tr>
<td>Seasonal variation</td>
<td>Cho et al., 1995; Hou and Yan, 1998; Martinez and Rico, 2002; Villares et al., 2002, 2013</td>
</tr>
<tr>
<td>Processing methods</td>
<td>Yoshie et al., 1994; Bermejo-Barrera et al., 2013</td>
</tr>
<tr>
<td>Method of mineralization</td>
<td>Fleurence &amp; LeCoeur, 1993; Fleurence, 1999</td>
</tr>
</tbody>
</table>

For example, a study by Muniz et al. (2008) compared the mineral contents of nori and konbu from Japan and Europe. Interestingly, Asian tested seaweeds presented higher levels of Pb and Cd (ng per gram dry matter) than their European counterparts. The authors suggested that this variation is possibly due to different levels of environmental pollution. Cho et al. (1995) documented that the trace mineral content of Korean seaweeds differed among sites, and fluctuated monthly at each site. Ruperez (2002) and van Netten et al. (2000) have also all reported a wide variation in mineral concentration in different marine algae species, even when differences in environmental factors were minimized.

**Health risks due to mineral toxicity**

Though seaweed consumption is steadily increasing in Western countries, few studies have reported on the potential toxicological health effects of habitual consumption. Minerals such as Ca, Fe, Mg, Se, Cu, or I are examples of essential minerals, whereas
heavy metals such as arsenic and lead are of great concern because of their excessive concentration in certain algae. Heavy metals are often detected in seaweeds due to their ability to bio-accumulate elements present in the water (Bocanegra et al., 2003; Besada et al., 2009; Oliveira et al., 2009) In some cases, seaweeds have even been used as biomarkers for marine pollution in coastal waters (Topcuoglu et al., 2003; Melville and Pulkownik, 2006).

Seaweed has been used as a source of iodine, however, some seaweed species contain levels of iodine that exceed the tolerable upper intake level of 1100 ug/d. This may lead to hypertension in some individuals if not carefully monitored (Teas et al., 2004).

However, heavy metal concentrations among seaweeds are highly dependent on various physiological and environmental factors (temperature, macronutrient concentrations, salinity, etc.) that affect their accumulation capacity (Küpper et al., 1998). In addition, because many of these factors are species specific, it is difficult to make any general extrapolations. In fact, inclusion of nori and wakame (7%) into the diets of rats showed no effect on organ weight or function, no histological effect, and a significant increase in fecal weight as a result of higher dietary fiber contents (Goni and Urbano, 2002; Bocanegra et al., 2003). Also, Ruperez (2002) reported heavy metal levels well below the toxic limits set forth by the United States Food and Nutrition Board (40ppm heavy metal limit).

**Mineral Bioavailability in Seaweeds & Influencing Factors**

Mineral bioavailability is expressed as the proportion of minerals present in the algae that can be utilized for essential body functions. The bioavailability of iron and other
minerals is diverse and differs significantly between species. Moredo-pinerio et al., (2012) evaluated the trace mineral bioavailability of edible seaweed utilizing an *in vitro* digestion approach coupling the use of a dialysis membrane with simulated intestinal digestion to measure mineral dializability. Their results demonstrated a large range of values, with high dialyzability percentages for Al, Fe, Mn, and Zn. The authors attributed the discrepancies to the varying nutritional composition of the samples. Shaw and Liu (2000) observed high levels of iron bioavailability of Nori using a hemoglobin regeneration assay. Similarly, Li et al. (2011) have also reported high bioavailability percentages for minerals Fe and Zn from *Laminaria* spp after an in vitro digestion and a biomimetic membrane extraction.

The bioavailability of seaweed minerals depends on the presence of other dietary factors that may affect mineral uptake (Table 1.9).

**Table 1.9- Factors that affect mineral bioavailability in seaweeds**

<table>
<thead>
<tr>
<th>Factors that affect bioavailability</th>
<th>Literature Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seaweed composition</td>
<td></td>
</tr>
<tr>
<td>Proteins</td>
<td>Garcia-Sartal et al., 2013</td>
</tr>
<tr>
<td>Dietary fiber</td>
<td>Garcia-Sartal et al., 2013</td>
</tr>
<tr>
<td>Polyphenolic compounds</td>
<td>Wang et al., 2009</td>
</tr>
<tr>
<td>Phytate</td>
<td>Fairweather-Tait and Hurrell, 1996</td>
</tr>
<tr>
<td>Chemical form of mineral</td>
<td>Fairweather-Tait and Hurrell, 1996</td>
</tr>
<tr>
<td>Cooking &amp; processing</td>
<td>Gupta et al., 2011; Bermejo-Barrera et al., 2013</td>
</tr>
</tbody>
</table>

The seaweed food matrix composition may interfere with the bioavailability of iron or other nutrients. Many dietary components inherent in seaweeds may react with minerals and form highly stable complexes that may decrease iron uptake. Algae are rich in a wide variety of minerals, but they are also rich in other components such as dietary fiber that may pass through the intestine without being absorbed and can also retain dietary mineral
components. In a study by Bermejo-Barrera et al. (2013), dietary fiber showed a statistically positive correlation with metal concentration in seaweeds. A negative association was seen between trace metal concentrations and fat and carbohydrate contents although this is element dependent (Bermejo-Barrera et al., 2013). Furthermore, the literature has reported both negative (Moreda-Pineiro 2012) and positive (Bermejo-Barrera et al., 2013) correlations of metal bioavailability with protein content in raw seaweeds. The negative correlation is attributed to the “salting out” effect caused by the ionic strength of the amino acids in the protein. This polarity inadvertently lowers the solubility of metals.

Polyphenolic compounds are a large and diverse group of naturally occurring compounds that include tannins, phenolic acids and flavonoids (Stern et al., 1996). Among the three seaweed classifications, brown algae generally contain higher amounts of polyphenols than red and green algae (Wang et al., 2009) Phlorotannins, a sub classification of tannins, are abundant at high levels in brown algae and their ability to form strong complexes with proteins and minerals is well documented (Stern et al. 1996). Studies have reported the significant scavenging effect and antioxidant activity in various brown algae species (Heo et al., 2005). High polyphenol contents could inhibit mineral absorption into the enterocyte by the formation of insoluble complexes.

Cooking and processing techniques also affect the bioavailability of minerals. Cooking processes promote the release of metals from seaweed matrices. Processing may improve mineral bioavailability via separation, partitioning or destroying inhibitors thereby enhancing mineral absorption. Iron retention after cooking was between 70-90% in Wakame and Konbu (brown seaweeds), Nori (red seaweed), and Sea Lettuce (green
seaweed) (Bermejo-Barrera et al., 2013). Processing can also exert negative effects on bioavailability by either 1) deactivating enzymes that degrade inhibitors or 2) producing insoluble compounds via oxidation or precipitation (Watzke, 1998).

In summary, seaweeds represent a nutrient-dense, low energy, food group. Edible seaweeds have been shown to contain essential vitamins and minerals, at levels that would augment a balanced diet if consumed regularly. Specifically, trace elements and minerals are abundant in seaweeds compared to terrestrial foods, and their versatile nature allows them to be used in many food and industrial products. Although seaweeds have been regarded as nutrient dense plant sources, they are not typically consumed in Western societies.

**THESIS OBJECTIVES**

The objectives of this thesis were to: 1) evaluate the iron content in commonly eaten seaweeds and seaweeds consumed in Hawaii 2) assess the iron bioavailability of selected seaweeds relative to boiled spinach and 3) based on the DRIs and recommended seaweed servings, determine which of the selected alga are good plant sources of iron. Seaweeds were chosen for this iron bioavailability study because of their purported high iron content, increasing consumption rates in Western culture, and their significance to the Hawaiian culture both economically and ethnically. Spinach was chosen as a relative comparison for iron bioavailability because it is commonly recognized as a dietary plant source of iron.


2.1. INTRODUCTION

Despite ongoing efforts to control its prevalence, iron deficiency remains the most common nutrient deficiency in the world affecting both developing and industrialized countries (WHO 2011). This is due in part to habitual low dietary iron intake or consumption of foods with low iron bioavailability (e.g., grains, vegetables, etc.) (WHO 2001; Polin et al., 2013). Given the current recommendations to increase the amount and variety of protein sources and plant foods (Dietary Guidelines, USDA and DHHS, 2010), there is an expected decrease in heme-iron intake and an increase in intake of the less absorbable non-heme iron. Additionally dietary changes would likely increase inhibitors of iron absorption such as phytates, oxalates, polyphenols and dietary fiber. These changes will decrease dietary iron bioavailability and may increase the prevalence of iron deficiency. For example, in the 25th release of the USDA National Nutrient Database for Standard Reference, boiled spinach is listed as one of the top plant food sources of iron. According to this compilation, 1 cup (180 g) of boiled, drained spinach contains 6.43 mg Fe (11.6 mg/100g). However, previous studies have demonstrated that the amount of iron actually absorbed (bioavailable iron) is well below this amount with as low as 1.4% of the iron actually being absorbed (Scrimshaw 1991). Therefore, to reduce the prevalence of iron deficiency, there exists a need to find novel plant food sources that are good sources of bioavailable iron.

Marine algae represent a natural food rich in iron and other minerals (Ortega-Calvo et al., 1993; MacArtain 2007). However, to our knowledge, the bioavailability of
iron from seaweeds has not been heavily investigated and this information is necessary to evaluate seaweeds as a potential source of bioavailable iron. For this reason, we sought to evaluate the relative iron bioavailability from common and Hawaiian seaweeds in comparison to boiled spinach.

To measure iron bioavailability we used the in vitro digestion/Caco-2 cell model of Glahn et al. (1998) to mimic intestinal digestion and absorption. Caco-2 cells are widely used and represent a valuable tool for approximation of intestinal iron absorption (Glahn et al., 1998). Mature caco-2 cells display features similar to normal, mature intestinal cells with respect to iron absorption and metabolism (Yun et al., 2004).

The objectives of this study were to: 1) determine the iron content of some commonly eaten seaweeds and local seaweeds found in Hawaii; and 2) assess the iron bioavailability of these seaweeds relative to spinach using an in vitro digestion/ Caco-2 cell model.

2.2. MATERIALS AND METHODS

2.2.1. Sample collection

In this study, ten seaweeds were investigated for their iron content: aonoriko, codium, dulse (powder and flake), gorilla ogo, green ogo, 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 from Maine Coast Sea Vegetables; Franklin, ME (see Table 2.1). Spinach was obtained fresh from a local grocery store in Honolulu, HI. Fresh seaweeds and spinach were processed within 24 hours of purchase.
Table 2.1- General characteristics of seaweeds analyzed

<table>
<thead>
<tr>
<th>Common Name(s)</th>
<th>Classification</th>
<th>Scientific Name Huisman et al., 2007</th>
<th>Hawaiian name Huisman et al., 2007</th>
<th>Geographic origin</th>
<th>Source</th>
<th>Sample State</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aonoriko</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Codium</td>
<td>Green algae</td>
<td><em>Codium edule</em></td>
<td>limu wawae’iole</td>
<td>Hawaiian Islands</td>
<td>Marukai (Honolulu, HI)</td>
<td>Fresh</td>
</tr>
<tr>
<td>Gorilla ogo</td>
<td>Red algae</td>
<td><em>Gracilaria salicornia</em></td>
<td>n/a</td>
<td>Hawaiian Islands</td>
<td>Marukai</td>
<td>Fresh</td>
</tr>
<tr>
<td>Green ogo</td>
<td>Red algae</td>
<td><em>Gracilaria parvispora</em></td>
<td>n/a</td>
<td>Kona, HI</td>
<td>Safeway (Honolulu, HI)</td>
<td>Fresh</td>
</tr>
<tr>
<td>Hijiki</td>
<td>Brown algae</td>
<td><em>Hizikia fusiformis</em></td>
<td>n/a</td>
<td>Japan</td>
<td>Maruya</td>
<td>Dry strips</td>
</tr>
<tr>
<td>Nori, Laver</td>
<td>Red algae</td>
<td><em>Porphyra sp.</em></td>
<td>limu lu’au or limu pahe’e</td>
<td>China</td>
<td>Shirokiya (Honolulu, HI)</td>
<td>Dry sheets</td>
</tr>
<tr>
<td>Red ogo</td>
<td>Red algae</td>
<td><em>Gracilaria coronopifolia</em></td>
<td>limu manauea</td>
<td>Hawaiian Islands</td>
<td>Marukai (Honolulu, HI)</td>
<td>Fresh</td>
</tr>
<tr>
<td>Rockweed</td>
<td>Brown algae</td>
<td><em>Ascophylum nodosum</em></td>
<td>n/a</td>
<td>Gulf of Maine (Bay of Fundy)</td>
<td>Maine Coast Sea Vegetables (seaveg.com)</td>
<td>Dry powder</td>
</tr>
<tr>
<td>Sea lettuce</td>
<td>Green algae</td>
<td><em>Ulva lactuca</em></td>
<td>n/a</td>
<td>Gulf of Maine (Bay of Fundy)</td>
<td>Maine Coast Sea Vegetables (seaveg.com)</td>
<td>Dry leaf and powder</td>
</tr>
<tr>
<td>Wakame</td>
<td>Brown algae</td>
<td><em>Undaria pinnatifida</em></td>
<td>n/a</td>
<td>Japan</td>
<td>Shirokiya (Honolulu, HI)</td>
<td>Dry strips</td>
</tr>
</tbody>
</table>
2.2.2. Sample preparation

Fresh Spinach: As spinach is usually consumed with leaves only, stalks and stems were removed before weighing and then rinsed with deionized water and pat dry. Spinach leaves were then boiled for 10 minutes in a stainless steel pot using deionized water, after which they were immediately removed and placed on ice for up to five minutes to prevent further cooking. The spinach was then drained for five minutes to remove any residual water. Spinach samples were homogenized in a Cuisinart food processor with stainless steel blades (previously acid washed in 10% HCl and rinsed with deionized water).

Spinach samples were lyophilized (VirTis Virtual 50xl, SP Scientific) using a 36-hour drying cycle. A temperature probe was placed into one representative sample during lyophilization to confirm complete dryness. Samples were stored in sealed plastic tubes and kept in a dessicator at room temperature until analyzed for mineral content and iron bioavailability.

Seaweed Sample Preparation: Within 24 hours of purchasing, fresh seaweeds were thoroughly washed three times in deionized water. Any visible invertebrates, sand or debris were removed. Seaweed was divided into 150 to 200 g portions and spun in a salad spinner for 30 s to remove excess water, and then weighed on a top-loading balance to 0.01g (wet weight). Fresh seaweed and dry seaweed products were placed on acid washed pyrex plates and dried to a constant weight at 50°C in an air oven. Dried samples were then ground into a fine powder using a Black and Decker coffee grinder with stainless steel bowl and blade. Samples were stored in plastic vials in a dessicator until further analysis.
2.2.3 Mineral analysis by ICPMS

Three, one-gram samples of each dried seaweed were sent to Louisiana State University Agricultural Center (LSU) for analysis of 17 minerals: aluminum, arsenic, boron, calcium, copper, lead, iron, magnesium, manganese, molybdenum, nickel, phosphorus, potassium, selenium, sodium, sulfur and zinc. Briefly, 0.5 gram aliquots of each sample were used for analysis; 5 ml concentrated trace metal grade HNO₃ was added to each aliquot and digested for 50 minutes using an automated block digester (Thomas Cain Inc. DEENA, Omaha, Nebraska). Then, 3 ml of H₂O₂ was added and further digested for 2.75 hours. The final mixture was cooled and diluted to 20 ml final volume. The mixture was then filtered through a 1-micron filter and analyzed using inductively coupled plasma-atomic emission spectroscopy (ICP-AES) (Spectro Arcos EOP ICAP, Germany). National Institutes of Standards and Technology (NIST) peach samples were digested and analyzed every 20 samples as a quality control. Dry matter mineral content obtained from LSU was expressed as µg per g dry matter.

2.2.4. Chemicals, Enzymes and Reagents

Unless otherwise stated, all chemicals and enzymes were purchased from Sigma Chemical (St. Louis, MO). All glassware utilized throughout these experiments was washed overnight in a 10% HCl/ 10% HNO₃ acid bath, and rinsed with deionized water before use. All the water used in experiments was cell culture grade, deionized water.
2.2.5. Cell Culture

Caco-2 cells were obtained from the American Type Culture Collection (Rockville, MD) at passage 17. Stock cell cultures were seeded at a density of 1,000,000 cells in 25 cm² plastic cell culture flasks and cultured in DMEM [Dulbecco’s Modified Eagle Medium/High Glucose containing 4.5 g/L glucose, 4.00 mM L-glutamine, 25 mM HEPES] and-supplemented with 10% v/v fetal bovine serum, (Hyclone, Logan, UT). The cells were maintained in an incubator at 37°C, in a 5% CO₂/95% air atmosphere (New Brunswick Scientific). The medium was replaced every 2 days. At 70-80% confluency, cells were detached from the flasks with 0.5% trypsin-EDTA (MediaTech) and reseeded in new 75 cm² plastic flasks. This subculturing procedure was continued until enough cells were accumulated to use in digestion experiments between passages 29-36.

After sufficient numbers of cells were obtained, the Caco cells were seeded onto collagen coated, polystyrene, 6-well tissue-culture plates (BD Biosciences). To collagen coat each plate, 9.47 mg/ml rat tail Type-1 collagen (BD Biosciences) was diluted with 0.02N acetic acid to a working concentration of 50 ug/ml. A 1.5ml aliquot of the collagen working solution was added to each well and incubated in a laminar flow hood for one hour. After one hour, the collagen working solution was removed, and the wells were rinsed with 2 ml Hank’s Buffered Salt Solution (HyClone). Each well was then seeded with Caco-2 cells at a density of 50,000 cells/cm². Cells were cultured in 2ml DMEM as described previously until 100% confluent monolayers were formed. Media was replaced every other day and after 12-16 days post-confluence, the cells were used in digestion experiments.
2.2.6. Bioavailability Experiment- Day 1

Preparation of 6-well culture plates

To measure relative iron bioavailability we employed an *in vitro* digestion/ Caco-2 cell model developed by Glahn et al (1998). One day prior to digestion, this model was set up as shown in Figure 2-1. Firstly, the DMEM was removed from each well, and then rinsed and replaced with 2 ml of freshly prepared MEM (Minimal Essential Media, HyClone), pH 7 supplemented with 10% FBS and 1% v/v antibiotic-antimycotic solution (pH 7.0).

To prepare the 6-well plates for experimentation, strips of metal free dialysis membrane (15 kDa MW cut-off, Membrane Filtration Products, Inc, Seguin, TX) were fitted onto 24 mm diameter Transwell plastic inserts (Costar) and fastened with silicon O-rings (Web Seal Inc, Rochester NY). The completed inserts were stored in deionized water at 4°C overnight. On the day of the experiment, the inserts were sterilized with 0.5 M HCl for one hour, rinsed and stored in deionized water until use.

![Diagram](image)

**Figure 2-1:** The *in vitro* digestion/ Caco-2 cell model (from Glahn et al. 1998)
2.2.7. Bioavailability Experiment- Day 2

All digestion reagents were freshly prepared on the day of the experiment, shortly before use as described below.

**Preparation of digestion reagents**

The pepsin solution was prepared by dissolving 0.8 g porcine pepsin in 20 ml of 0.1 M HCl. Once mixed, 10 grams of Chelex-100 (Chelex-100 sodium form) was added to the solution and gently stirred on a tabletop shaker (New Brunswick Scientific) at 125 revolutions/min for 45 minutes. The chelexed slurry was then poured into a 1.6 cm diameter filtration column to remove the resin and collect the eluant. The column was further eluted with an additional 20 ml of 0.1 M HCl for a final volume of 40 ml pepsin solution.

To prepare the pancreatin-bile solution, 0.3 g pancreatin and 1.8 g bile extract were dissolved in 150 ml of 0.1 M NaHCO₃. Similar to the above procedure, 75 g of Chelex-100 was added to this solution and gently mixed on a tabletop shaker at 125 revolutions/min for 30 minutes. The slurry was poured into a 1.6 cm diameter filtration column to collect the eluent and filter the Chelex. The column was further eluted with an additional 60 ml of 0.1 M NaHCO₃ for a final volume of 210 ml pancreatin-bile solution.

**Preparation of quality control samples:**

For the digestion experiments, three quality controls were utilized: a blank sample, a ferrous sulfate (FeSO₄) sample, and an FeSO₄ + Vitamin C sample. The blank sample, contained only the digest solutions and served to demonstrate a low baseline for the formation of ferritin from the trace amounts of iron innately present in the digest
solutions and culture medium. The FeSO$_4$ treatment functioned as a positive control and was used to demonstrate the increase in ferritin formation upon addition of 55µg FeSO$_4$ to the blank digest (67 µM iron). The 55 µg FeSO$_4$ amount was chosen because maximum sensitivity of iron uptake in the Caco-2 cell *in vitro* system has been previously demonstrated at this level (Glahn et al. 1998). The FeSO$_4$ + Vitamin C treatment was used as a positive control to show that the Caco-2 cells respond to the enhancing effects of vitamin C on iron bioavailability.

In order to make the ferrous sulfate quality control working sample, first an iron stock solution was made by dissolving 0.0556 g FeSO$_4$ 7H$_2$O (FW = 278.01) to 10 ml with 1% sulfuric acid (H$_2$SO$_4$). A 400 µl aliquot of this iron stock solution was then added to 3.6 ml of 1% H$_2$SO$_4$ to produce a working iron solution. Finally, 500 µl of the working iron solution was added to 15 mL of digest solution resulting in a final concentration of 67 µM iron.

Ferrous sulfate + vitamin C positive control samples were derived from a vitamin C stock solution. This stock solution (200mM) was made by adding 0.3522 g ascorbic acid (FW = 176.1) (Macron Fine Chemicals) to 10 ml of deionized water after which the solution was covered to protect from light. Then, 800 µl of the vitamin C stock was added to 400 µl of the iron stock described above and 2.8 ml of 1% H$_2$SO$_4$. After stirring this solution was kept in the dark until needed. When ready, 500 µl of this working ferrous sulfate + vitamin C solution was added to 15 mL of digest solution to produce a final concentration of 1.3 mM Vitamin C and 67 µM Fe.
Preparation of digests and exposure to cell monolayers:

The in vitro digestions for each sample were carried out in 50-ml conical “digest” tubes. Dried, ground samples of spinach and seaweeds were weighed to 0.5 grams and placed in weighed digest tubes. Therefore, equal amounts of the plant foods were used (0.5 g DM), but the amounts of iron in the digests varied (see table 1 in Appendix). Next, 10 ml of a 140 mM NaCl, 5 mM KCl (pH 2.0) salt solution was added to the tubes. For the positive quality controls (FeSO$_4$ and FeSO$_4$ + Vit C samples), 500μl of the working solution was added to tubes containing the 10 ml salt solution. All tubes were then readjusted to pH 2 with 0.1 M HCl after which 0.5 ml pepsin solution was added. All digest tubes were then vortexed, and placed horizontally on a shaker set at 60 rpm and incubated for 1 hour at 37°C. During the 1-hr incubation of the digests, MEM was removed from the 6-well plates, and replaced with 1 ml fresh MEM. The inserts were then fitted onto each well and the plates were returned to the incubator until needed.

Immediately following the 1-hr incubation, the pH of the digests was adjusted to 5.5-6.0 with 1 M NaHCO$_3$. Then, 2.5 ml of pancreatin-bile solution was added to each digest tube, mixed, and the pH was adjusted to pH 6.9-7.0. Finally, the total volume of each digest was standardized to 15 ml by the dropwise addition of 140 mM NaCl, 5 mM KCl (pH 6.7). The digests were gently vortexed and a 1.5 ml aliquot was placed on top of the dialysis membrane in the insert of a well in the culture plate. The plates were gently rocked in the incubator for two hours, after which the inserts were removed from each well and a further 1 ml of fresh MEM was added. The plates were then returned to the incubator for a further 16 hours until harvesting.
2.2.8. Bioavailability Experiment- Day 3

After an incubation of 18 hours, the MEM was removed from all plates and the cells were rinsed twice with 2 ml of a 130 mM, 5 mM KCl, 5 mM PIPES (pH 6.7) buffer solution. The cells were then harvested by adding 2 ml of deionized water to each well, followed by the subsequent scraping of the cells off the plastic substrate surface with a transfer pipette. The cells (suspended in 2 ml of deionized water) were then transferred to sterile 5 ml tubes. The harvested cells were sonicated on high for 15 minutes using a bench top sonicator. Sonicated cells were stored at -20°C until analyzed for cell protein and ferritin content.

2.2.9. Experimental Design

Four different experiments were conducted, all of which are listed in Table 2-2. In each experiment, three to six digests were prepared for each food sample (n = 3-6) and each digest was randomly placed into a well on a separate 6-well plate. For experiment 1 only, three trials were conducted, each trial using a different subculture of Caco-2 cells.

Experiment 1 evaluated the bioavailability of 3 different seaweed samples (red ogo, nori and sea lettuce) relative to boiled spinach. Experiment 2 evaluated the bioavailability of rockweed, wakame, and wawae’iole relative to spinach. Experiment 3 assessed the effects of vitamin C (a potent enhancer of iron absorption) on iron bioavailability from spinach and the same seaweeds evaluated in Experiment 1. This was done by adding a physiological dose of vitamin C to each food digest so that the final concentration of vit C in each digest tube was 0.28 mM. Experiment 4 looked at the presence of inhibitors in the same three seaweeds by adding 0.28 mM Vitamin C and an
additional 55µg iron (as FeSO₄) to the 15 mL digest. With the addition of more iron, there would be an expected increase in bioavailable response unless inhibitors were inherently present in the seaweed itself.

Table 2-2. List of all experiments conducted and their design

<table>
<thead>
<tr>
<th>Experiment #</th>
<th>Objective</th>
<th>Samples</th>
<th>No. of Trials</th>
<th>Independent Variable</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experiment 1</td>
<td>Bioavailability comparison</td>
<td>Red ogo, nori, sea lettuce &amp; spinach</td>
<td>3</td>
<td>n/a</td>
</tr>
<tr>
<td>Experiment 2</td>
<td>Bioavailability comparison</td>
<td>Rockweed, wakame, wawae’iole &amp; spinach</td>
<td>1</td>
<td>n/a</td>
</tr>
<tr>
<td>Experiment 3</td>
<td>Effect of enhancers (Vitamin C) on bioavailability</td>
<td>Red ogo, nori, sea lettuce &amp; spinach</td>
<td>1</td>
<td>0.1 g Vitamin C / 2L [0.00028 M]</td>
</tr>
<tr>
<td>Experiment 4</td>
<td>Presence of inhibitors</td>
<td>Red ogo, nori, &amp; sea lettuce</td>
<td>1</td>
<td>FeSO₄</td>
</tr>
</tbody>
</table>

2.2.10. Chemical Analyses

Cell protein assay

Cell protein concentrations were measured using the Modified Lowry Protein Assay Kit (Thermo Scientific). Assays were done in triplicate using 200 µl aliquots of the harvested, sonicated cells. Prior to analysis, sonicated cell samples were defrosted in cold water and reagents were brought to room temperature. When not in use, cell samples were kept on ice during analysis. Aliquots (200 µl) of cell lysate were placed in 5 ml disposable culture tubes (VWR). Then, 1.0 ml Modified Lowry Reagent was added to each tube and mixed for 15 seconds. This was followed by the addition of 100 µl of prepared 1x Folin-Ciocalteu Reagent was added and mixed for 15 seconds. The samples were covered and incubated in the dark for 30 minutes. After incubation the absorbance
of each sample was recorded at 750 nm (UV Visible Spectrophotometer, Shimadzu). A standard curve was generated at BSA concentrations of 0, 1, 5, 25, 125, 250, 500, 750, and 1000 μg/ml using the same steps as the cell lysate.

**Cell ferritin assay**

Ferritin concentrations were quantitatively measured in duplicate using an enzyme-linked immunosorbent assay (ELISA) following manufactures instructions (Spectro Ferritin MT kit, Ramco Laboratories Inc., Houston, TX). Prior to analysis, the cell samples were defrosted in cold water and reagents were brought to room temperature. When not in use, cell samples were kept on ice during analysis. Aliquots of 10 μl of the sonicated cells were added to pre-assigned wells in a 96 well plate. Then, 200 μl of conjugated antihuman ferritin was placed into each well and the plate was incubated on a platform shaker at 190 rpm for 2 hours. After incubation, the plate was washed 3 times with deionized water and 200 μl of substrate solution was added to each well. The plate was further incubated for 30 minutes, after which 100 μl of 0.24% potassium ferricyanide was added to each well. Finally, the plate was shaken at 190 rpm for 1 minute to develop the color and absorbance was read at 510 nm using a microplate reader (BioTek Instruments). A ferritin standard curve was generated using concentrations of 0, 6, 20, 60, 200, 600, 2000 ng/ml.

**2.2.11. Calculations and Statistics**

Bioavailability of each sample was calculated as ng ferritin/ mg cell protein in each well containing 0.5 g dried food sample from the 1.5 ml of digest added. Efficiency of iron uptake from foods (efficiency of iron bioavailability) was calculated as ng ferritin
/ mg cell protein / μg Fe in each well. All data were expressed as means ± SD, n = 3-6 (see table and figures for details). When needed, data was log transformed to achieve equal variances prior to analyses. All statistical analyses were performed using the GraphPad Prism version 5 (GraphPad Software, San Diego, CA). A one-way ANOVA was performed followed by Tukey’s post hoc test to compare differences among means. Differences were considered significant if p ≤ 0.05.

2.3. RESULTS

2.3.1 Mineral Composition of Seaweeds

The mineral contents in the seaweed samples are shown in Tables 2.3 and 2.4. The range in values for each mineral varied from 7 to 150-fold between seaweeds. This serves as an indication to the diversity of minerals in each species and the wide range at which these minerals are found. For example, copper content was highest in aonoriko (0.31mg/ 10g DM). This is more than a 20-fold increase from the lowest copper containing seaweed, wawae’iole. In comparison to the other minerals evaluated, the variability in manganese content was also high with a 122-fold between-sample difference. Sodium content also varied greatly ranging from 95 mg/ 10 g DW (Nori) to 1914 mg/10g DW (wawae’iole).

Mineral nutrients

In general, most of the seaweeds followed similar trends in mineral composition and a three-tiered division between the minerals analyzed was observed. Sodium, iron and manganese were the three most dominant minerals present in most of the seaweeds,
respectively making up tier 1. Zinc, copper and potassium content followed in tier 2. Calcium and magnesium were interchangeably the lowest in all seaweeds, comprising the final tier 3.

The mineral nutrients to note are iron, manganese and sodium. The highest amount of iron was found in dulse powder and aonoriko with 35 and 17 mg Fe per 10 g dry weight, respectively. Sea lettuce (flake and leaf), rockweed and red ogo contained between 5-8 mg Fe / 10 g DW while all other seaweeds contained less than 5 mg. Furthermore, calcium, magnesium, molybdenum, and phosphorus contents were low in all seaweeds (≤ 0.03 mg/ 10g DW.)

The labeling of good and excellent sources of nutrients was established as a simple and understandable method for disseminating the nutritional content of a particular food to consumers. A nutrient content claim for a ‘good’ source is a food item that contains 10-19% of the Daily Value (DV) per Reference amounts customarily consumed per eating occasion (RACC). A claim for ‘excellent’ is 20% or more of the DV per RACC. The RACC, as established by the FDA, is the amount of food normally consumed per eating occasion by persons four years of age or older. In our study we have defined the RACC for seaweed as 10 g DW. As shown in Table 2.4, some seaweeds reached the level of a “good” or “excellent” source for 4 of the 11 mineral-nutrients analyzed: copper, iron, sodium, and manganese.

Aonoriko, Dulse powder, green ogo, nori, and rockweed were all considered excellent sources of manganese while gorilla ogo was a good source. Although red ogo also met the criteria as an excellent source of Mn, it exceeds the UL for children. Manganese is a mineral that is both nutritionally essential and potentially toxic if
consumed in excess. Currently, the Food and Nutrition Board of the Institute of medicine set the tolerable upper intake level (UL) for Mn at 3, 6, and 11 mg/day for toddlers (age 4-8 years), children (age 9-13 years) and adults (19 years and older), respectively (Food and Nutrition Board, 2001). As shown in Table 2.4, the Mn content in red ogo exceeds the UL for toddlers and children, and approaches half the upper limit for adults in a single 10g dry sample.

Except for gorilla ogo, green ogo and hijiki, all remaining seaweeds met or exceeded the FDA definition for good or rich sources of iron. Aonoriko and dulse powder exceeded the amount of iron considered as an excellent source by ~4x and 9x, respectively.

None of the seaweeds were good sources of the macro-minerals calcium, phosphorus, and potassium or the micro-minerals zinc. Aonoriko was the only seaweed that met the DV for copper. In addition, Hijiki was the only seaweed that did not meet the definitions for good or excellent sources in any of the minerals.
Table 2.3. Selected mineral composition of seaweeds expressed per 10g dry weight\(^1, 2, 3\)

<table>
<thead>
<tr>
<th>Name</th>
<th>Ca (µg)</th>
<th>Mg (µg)</th>
<th>K (µg)</th>
<th>Na (mg)</th>
<th>Cu (mg)</th>
<th>Fe (mg)</th>
<th>Mn (mg)</th>
<th>Zn (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aonoriko</td>
<td>4.89 ± 0.07</td>
<td>9.57 ± 0.13</td>
<td>26.96 ± 0.14</td>
<td>113.71 ± 0.66</td>
<td>0.31 ± 0.00</td>
<td>17.12 ± 0.39</td>
<td>0.40 ± 0.00</td>
<td>0.32 ± 0.01</td>
</tr>
<tr>
<td>Dulse leaf</td>
<td>1.76 ± 0.16</td>
<td>2.89 ± 0.02</td>
<td>95 ± 4</td>
<td>229 ± 2</td>
<td>0.05 ± 0.00</td>
<td>3.25 ± 0.59</td>
<td>0.13 ± 0.00</td>
<td>0.24 ± 0.00</td>
</tr>
<tr>
<td>Dulse powder</td>
<td>15.18 ± 0.93</td>
<td>4.91 ± 0.07</td>
<td>81.19 ± 0.41</td>
<td>244 ± 2</td>
<td>0.06 ± 0.00</td>
<td>34.90 ± 1.41</td>
<td>1.49 ± 0.00</td>
<td>0.37 ± 0.01</td>
</tr>
<tr>
<td>Gorilla ogo</td>
<td>3.74 ± 0.44</td>
<td>2.59 ± 0.08</td>
<td>201 ± 2</td>
<td>169 ± 5</td>
<td>0.04 ± 0.00</td>
<td>1.05 ± 0.06</td>
<td>0.32 ± 0.00</td>
<td>0.79 ± 0.02</td>
</tr>
<tr>
<td>Green ogo</td>
<td>2.25 ± 0.02</td>
<td>4.24 ± 0.04</td>
<td>155.35 ± 0.29</td>
<td>180 ± 2</td>
<td>0.01 ± 0.00</td>
<td>1.45 ± 0.01</td>
<td>0.46 ± 0.00</td>
<td>0.20 ± 0.00</td>
</tr>
<tr>
<td>Hijiki</td>
<td>11.96 ± 0.17</td>
<td>5.86 ± 0.04</td>
<td>63.31 ± 0.66</td>
<td>178 ± 2</td>
<td>0.01 ± 0.00</td>
<td>0.73 ± 0.04</td>
<td>0.13 ± 0.00</td>
<td>0.11 ± 0.00</td>
</tr>
<tr>
<td>Nori sheet</td>
<td>2.92 ± 0.03</td>
<td>4.29 ± 0.02</td>
<td>16.69 ± 0.17</td>
<td>95.4 ± 0.4</td>
<td>0.19 ± 0.00</td>
<td>2.71 ± 0.00</td>
<td>0.30 ± 0.00</td>
<td>0.27 ± 0.00</td>
</tr>
<tr>
<td>Red Ogo</td>
<td>1.94 ± 0.09</td>
<td>2.68 ± 0.22</td>
<td>136 ± 14</td>
<td>115 ± 13</td>
<td>0.05 ± 0.00</td>
<td>5.19 ± 0.55</td>
<td>5.69 ± 0.42</td>
<td>0.20 ± 0.00</td>
</tr>
<tr>
<td>Rockweed</td>
<td>29.04 ± 0.89</td>
<td>7.26 ± 0.26</td>
<td>20.82 ± 0.45</td>
<td>288 ± 8</td>
<td>0.02 ± 0.00</td>
<td>7.04 ± 0.24</td>
<td>0.40 ± 0.01</td>
<td>0.08 ± 0.00</td>
</tr>
<tr>
<td>Sea Lettuce flake</td>
<td>4.81 ± 0.18</td>
<td>24.74 ± 0.93</td>
<td>31.77 ± 1.60</td>
<td>355 ± 17</td>
<td>0.03 ± 0.00</td>
<td>8.31 ± 0.29</td>
<td>0.17 ± 0.00</td>
<td>0.08 ± 0.01</td>
</tr>
<tr>
<td>Sea lettuce leaf</td>
<td>4.58 ± 0.13</td>
<td>22.52 ± 0.16</td>
<td>33.42 ± 0.18</td>
<td>377 ± 2</td>
<td>0.03 ± 0.00</td>
<td>8.30 ± 0.16</td>
<td>0.18 ± 0.00</td>
<td>0.09 ± 0.00</td>
</tr>
<tr>
<td>Wakame</td>
<td>10.03 ± 0.05</td>
<td>6.99 ± 0.06</td>
<td>4.31 ± 0</td>
<td>788 ± 8</td>
<td>0.03 ± 0.00</td>
<td>3.82 ± 0.13</td>
<td>0.12 ± 0.00</td>
<td>0.44 ± 0.00</td>
</tr>
<tr>
<td>Wawae’iole</td>
<td>9.83 ± 0.49</td>
<td>15.61 ± 0.02</td>
<td>7.02 ± 0.22</td>
<td>1914 ± 11</td>
<td>0.01 ± 0.00</td>
<td>2.14 ± 0.38</td>
<td>0.10 ± 0.05</td>
<td>0.03 ± 0.00</td>
</tr>
</tbody>
</table>

\(^1\) Values are means ± SD (n=3).
\(^2\) Mean values for Molybdenum, Phosphorus and Selenium in all seaweed samples ≤ 0.005 mg/ 10 g DW
\(^3\) SD ≤ 0.005 were rounded to 0
Table 2.4. Selected mineral composition of seaweeds expressed as percent of Daily Value

<table>
<thead>
<tr>
<th></th>
<th>Cu %</th>
<th>Fe %</th>
<th>Na %</th>
<th>Mn %</th>
<th>Zn %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Daily Value (DV)</td>
<td>2.0 mg</td>
<td>18 mg</td>
<td>2,400 mg</td>
<td>2.0 mg</td>
<td>15 mg</td>
</tr>
<tr>
<td>Excellent (20% of DV) **</td>
<td>0.4 mg</td>
<td>3.6 mg</td>
<td>480 mg</td>
<td>0.4 mg</td>
<td>3.0 mg</td>
</tr>
<tr>
<td>Good (10-19% of DV) *</td>
<td>0.2 mg</td>
<td>1.8 mg</td>
<td>240 mg</td>
<td>0.2 mg</td>
<td>1.5 mg</td>
</tr>
<tr>
<td>Aonoriko</td>
<td>16%*</td>
<td>95%**</td>
<td>5%</td>
<td>20%**</td>
<td>2%</td>
</tr>
<tr>
<td>Dulse leaf</td>
<td>3%</td>
<td>18%*</td>
<td>9.5%</td>
<td>7%</td>
<td>2%</td>
</tr>
<tr>
<td>Dulse powder</td>
<td>3%</td>
<td>194%**</td>
<td>10%*</td>
<td>75%**</td>
<td>3%</td>
</tr>
<tr>
<td>Gorilla ogo</td>
<td>2%</td>
<td>6%</td>
<td>7%</td>
<td>16%*</td>
<td>5%</td>
</tr>
<tr>
<td>Green ogo</td>
<td>0.5%</td>
<td>8%</td>
<td>8%</td>
<td>23%**</td>
<td>1%</td>
</tr>
<tr>
<td>Hijiki</td>
<td>0.5%</td>
<td>4%</td>
<td>7%</td>
<td>7%</td>
<td>0.7%</td>
</tr>
<tr>
<td>Nori sheet</td>
<td>10%*</td>
<td>15%*</td>
<td>4%</td>
<td>15%**</td>
<td>2%</td>
</tr>
<tr>
<td>Red Ogo</td>
<td>3%</td>
<td>29%**</td>
<td>5%</td>
<td>285%**</td>
<td>1%</td>
</tr>
<tr>
<td>Rockweed</td>
<td>1%</td>
<td>39%**</td>
<td>12%*</td>
<td>20%**</td>
<td>0.5%</td>
</tr>
<tr>
<td>Sea Lettuce flake</td>
<td>2%</td>
<td>46%**</td>
<td>15%*</td>
<td>9%</td>
<td>0.5%</td>
</tr>
<tr>
<td>Sea lettuce leaf</td>
<td>2%</td>
<td>46%**</td>
<td>16%*</td>
<td>9%</td>
<td>0.6%</td>
</tr>
<tr>
<td>Wakame</td>
<td>2%</td>
<td>21%**</td>
<td>33%**</td>
<td>6%</td>
<td>3%</td>
</tr>
<tr>
<td>Wawae’iole</td>
<td>0.5%</td>
<td>12%*</td>
<td>80%**</td>
<td>5%</td>
<td>0.2%</td>
</tr>
</tbody>
</table>

1 Values are means ± SD (n=3). % Daily Value (DV) was calculated as [(mg/10g dry weight) divided by mineral DV (mg)]* 100 = %DV
* Good source based on DV’s. DV’s are based on a 2000-calorie diet for adults and children four years of age and up.
** Excellent source based on DV’s. DV’s are based on a 2000-calorie diet for adults and children four years of age and up.
Table 2.5. Non-nutrient mineral composition of seaweeds expressed as mg per 10g dry weight\(^1,2,3\)

<table>
<thead>
<tr>
<th>Name</th>
<th>Al (mg)</th>
<th>As (µg)</th>
<th>B (mg)</th>
<th>Ni (µg)</th>
<th>S (µg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aonoriko</td>
<td>18.45 ± 0.64</td>
<td>67 ± 2</td>
<td>1.2 ± 0.01</td>
<td>118 ± 2.33</td>
<td>23 ± 5.5</td>
</tr>
<tr>
<td>Dulse leaf</td>
<td>1.96 ± 0.36</td>
<td>148 ± 2</td>
<td>1.7 ± 0.02</td>
<td>48 ± 3.5</td>
<td>6 ± 0.05</td>
</tr>
<tr>
<td>Dulse powder</td>
<td>24.96 ± 1.19</td>
<td>120 ± 2</td>
<td>1.6 ± 0.1</td>
<td>79 ± 1.8</td>
<td>8 ± 0.09</td>
</tr>
<tr>
<td>Gorilla ogo</td>
<td>0.41 ± 0.04</td>
<td>91 ± 2</td>
<td>4.0 ± 0.06</td>
<td>12 ± 1.6</td>
<td>29 ± 0.54</td>
</tr>
<tr>
<td>Green ogo</td>
<td>0.17 ± 0.00</td>
<td>144 ± 2</td>
<td>2.5 ± 0.05</td>
<td>16 ± 0.21</td>
<td>39 ± 0.41</td>
</tr>
<tr>
<td>Hijiki</td>
<td>0.72 ± 0.06</td>
<td>666 ± 2</td>
<td>1.4 ± 0.09</td>
<td>17 ± 0.77</td>
<td>13 ± 0.12</td>
</tr>
<tr>
<td>Nori sheet</td>
<td>2.12 ± 0.05</td>
<td>224 ± 3</td>
<td>0.24 ± 0.0</td>
<td>12 ± 0.2</td>
<td>22 ± 0.17</td>
</tr>
<tr>
<td>Red Ogo</td>
<td>2.47 ± 0.41</td>
<td>51 ± 3</td>
<td>2.7 ± 0.05</td>
<td>124 ± 9</td>
<td>36 ± 2.6</td>
</tr>
<tr>
<td>Rockweed</td>
<td>3.55 ± 0.07</td>
<td>273 ± 8</td>
<td>1.1 ± 0.06</td>
<td>17 ± 0.76</td>
<td>22 ± 0.69</td>
</tr>
<tr>
<td>Sea Lettuce flake</td>
<td>6.33 ± 0.22</td>
<td>37.4 ± 0.9</td>
<td>0.50 ± 0.04</td>
<td>35 ± 1</td>
<td>39 ± 1.6</td>
</tr>
<tr>
<td>Sea lettuce leaf</td>
<td>6.53 ± 0.18</td>
<td>29 ± 1</td>
<td>0.60 ± 0.01</td>
<td>3 ± 2</td>
<td>38 ± 0.23</td>
</tr>
<tr>
<td>Wakame</td>
<td>2.44 ± 0.01</td>
<td>303 ± 6</td>
<td>0.3 ± 0.0</td>
<td>34 ± 0.2</td>
<td>8 ± 0.07</td>
</tr>
<tr>
<td>Wawae’iole</td>
<td>0.63 ± 0.03</td>
<td>90 ± 3</td>
<td>0.7 ± 0.04</td>
<td>10 ± 0.67</td>
<td>35 ± 2.6</td>
</tr>
</tbody>
</table>

\(^1\) Values are means ± SD (n=3)
\(^2\) Mean values for Cadmium and Lead in all seaweed samples ≤ 0.005 mg/10g DW
\(^3\) SD ≤ 0.005 were rounded to 0
Non-nutrient mineral content

The amounts of non-nutrient minerals in the seaweeds and their relationship to published toxicity levels are shown in Tables 2.5 and 2.6, respectively. Aluminum content reached as high as 24.9 mg Al/10g DM in dulse powder and 18.5 mg/10 g in aonoriko (Table 2.5). This is compared to the provisional tolerable weekly intake (PTWI) of 2 mg Al/kg body weight set by the FAO/WHO Joint Expert Committee on Aluminum-containing Food Additives (JECFA)(WHO 2011)(Table 2.6). Also of note was a correlation between iron content and aluminum content in the seaweeds. This is consistent with findings from Lai et al., (2009). This trend was seen despite large variations in Fe and Al contents, more than 48-fold and 150-fold, respectively.

Hijiki contained the highest amount of arsenic per gram DW (0.066 mg/g DW) compared to the other samples. This is consistent with findings from previous studies (Hanaoka 2001; Ichikawa 2006). A PTWI of 0.003 mg Ar/kg body weight set by the FAO/WHO Joint Expert Committee on arsenic-containing Food Additives (JECFA)(WHO 2011) (Table 2.6). Hijiki, dulse, nori, wakame and rockweed all contained potentially toxic levels of arsenic (Table 2.6).

Lead content was not detected in any of the seaweeds except for dulse powder and aonoriko which contained 0.003 and 0.001 mg/g DW, respectively. These values are in comparison to a PTWI of 0.025 mg Pb/kg body weight set by the FAO/WHO Joint Expert Committee on lead contaminants in foods (JECFA)(WHO 2011). Furthermore, cadmium and sulfur contents were low in all seaweeds (≤ 0.03 mg/10g DW.)
<table>
<thead>
<tr>
<th>Tolerable daily Intake (TDI)</th>
<th>Al %</th>
<th>As %</th>
<th>B %</th>
<th>Ni %</th>
<th>Pb %</th>
</tr>
</thead>
<tbody>
<tr>
<td>TDI for 70 kg male</td>
<td>0.28 mg/kg BW/day</td>
<td>2.1 µg/kg BW/day</td>
<td>0.2 mg/kw BW/day</td>
<td>11 µg/kg BW/day</td>
<td>3.6 µg/kg BW/day</td>
</tr>
<tr>
<td>Aonoriko</td>
<td>20 mg</td>
<td>147 µg</td>
<td>14 mg</td>
<td>770 µg</td>
<td>252 µg</td>
</tr>
<tr>
<td>Dulse leaf</td>
<td>92%</td>
<td>46%</td>
<td>9%</td>
<td>15%</td>
<td>6%</td>
</tr>
<tr>
<td>Dulse powder</td>
<td>10%</td>
<td>101%*</td>
<td>12%</td>
<td>6%</td>
<td>ND</td>
</tr>
<tr>
<td>Gorilla ogo</td>
<td>125%*</td>
<td>82%</td>
<td>11%</td>
<td>10%</td>
<td>14%</td>
</tr>
<tr>
<td>Green ogo</td>
<td>2%</td>
<td>62%</td>
<td>29%</td>
<td>2%</td>
<td>ND</td>
</tr>
<tr>
<td>Hijiki</td>
<td>0.9%</td>
<td>98%</td>
<td>18%</td>
<td>2%</td>
<td>ND</td>
</tr>
<tr>
<td>Nori sheet</td>
<td>4%</td>
<td>453%*</td>
<td>10%</td>
<td>2%</td>
<td>ND</td>
</tr>
<tr>
<td>Red Ogo</td>
<td>11%</td>
<td>152%*</td>
<td>2%</td>
<td>2%</td>
<td>ND</td>
</tr>
<tr>
<td>Rockweed</td>
<td>12%</td>
<td>35%</td>
<td>19%</td>
<td>16%</td>
<td>ND</td>
</tr>
<tr>
<td>Sea Lettuce flake</td>
<td>18%</td>
<td>186%*</td>
<td>8%</td>
<td>2%</td>
<td>ND</td>
</tr>
<tr>
<td>Sea lettuce leaf</td>
<td>32%</td>
<td>25%</td>
<td>4%</td>
<td>5%</td>
<td>ND</td>
</tr>
<tr>
<td>Wakame</td>
<td>3%</td>
<td>61%</td>
<td>5%</td>
<td>1%</td>
<td>ND</td>
</tr>
<tr>
<td>Wawae’iole</td>
<td>12%</td>
<td>206%*</td>
<td>2%</td>
<td>4%</td>
<td>ND</td>
</tr>
</tbody>
</table>

1 Values are means (n=3). ND = not detected
2 Provisional Tolerable Weekly Intakes (PTWIs) are defined as the acceptable level of toxic metal that can be ingested on a weekly basis per kg body weight (BW), as determined by the World Health Organization and the Food and Agriculture Organization (WHO 2010). For our purposes we have converted PTWI for Al, Ar and Pb to a tolerable daily intake by dividing each PTWI value by 7.
3 No PTWI, value based on recommendation for total dietary intake per day by Environmental Protection Agency (EPA) (US EPA, 2004a)
4 No PTWI, value based on recommendation for total dietary intake per week (WHO Guidelines for Drinking Water Quality, 2007) divided by 7.

* Exceeds PTWI
2.3.2. Bioavailability Studies

The amounts of total iron found in seaweed samples and spinach expressed as µg per gram dry weight (DW) are shown in Table 2.7. Seaweeds used for iron bioavailability experiments (nori, red ogo, rockweed, sea lettuce, wakame, and wawae’iole) were selected based on high iron content, frequency of consumption in Western diets, or significance in Hawai’i as found in the literature review in Chapter 1.

Table 2.7. Total iron content in seaweeds and spinach

<table>
<thead>
<tr>
<th>Plant Sample</th>
<th>µg/g, dry weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aonoriko</td>
<td>1712 ± 4</td>
</tr>
<tr>
<td>Dulse powder</td>
<td>3490 ± 15</td>
</tr>
<tr>
<td>Dulse leaf</td>
<td>325 ± 9</td>
</tr>
<tr>
<td>Gorilla ogo</td>
<td>105 ± 19</td>
</tr>
<tr>
<td>Green ogo</td>
<td>145 ± 3</td>
</tr>
<tr>
<td>Hijiki</td>
<td>73 ± 9</td>
</tr>
<tr>
<td>Nori sheet*</td>
<td>271 ± 0.3</td>
</tr>
<tr>
<td>Red ogo*</td>
<td>519 ± 10</td>
</tr>
<tr>
<td>Rockweed*</td>
<td>704 ± 3</td>
</tr>
<tr>
<td>Sea Lettuce flake</td>
<td>831± 15</td>
</tr>
<tr>
<td>Sea Lettuce powder*</td>
<td>830 ± 12</td>
</tr>
<tr>
<td>Wakame*</td>
<td>382 ± 1.2</td>
</tr>
<tr>
<td>Wawae’iole*</td>
<td>214 ± 6</td>
</tr>
<tr>
<td>Reference food</td>
<td></td>
</tr>
<tr>
<td>Spinach</td>
<td>166 ± 4</td>
</tr>
</tbody>
</table>

1 Values are means ± SD in triplicate. Means expressed in µg Fe per gram dry weight were compared using ANOVA followed by Tukey's post-hoc test. Means with different superscripts differ significantly (p ≤ 0.05).

* Foods chosen for in vitro digestion/Caco-2 cell bioavailability studies

Results for iron bioavailability in the quality control samples are shown as inserts in Figures 2.1A, 2.2A, 2.3 A and 2.4A. In all experiments, the blank samples showed a low baseline for ferritin formation from the trace amount of iron present in the digest reagents and MEM. The FeSO₄ digest was our positive control and demonstrated a
significant increase in ferritin formation upon the addition of 67 µmol of FeSO₄. The FeSO₄ + Vit C digests showed a further increase in bioavailable response, thus demonstrating that the Caco-2 system responded to the enhancing effect of 1.3 µM Vit C on iron bioavailability. The quality controls served as an indication that the Caco-2 cells in all experiments were well differentiated and were responding appropriately to the ferrous sulfate and vitamin C.

**Experiment 1: Red ogo, nori and sea lettuce compared to spinach**

Red ogo, nori and sea lettuce were selected for Experiment 1. In this experiment, 0.05 g of dry matter (DM) within 1.5 ml of digest was exposed to each well and iron bioavailability was measured as ng ferritin / mg cell protein per 0.05 g of dry matter (Figure 2.1A). The 0.05 g DM in each well contained varying amounts of iron for the different food samples (Table 1, Appendix). Compared to spinach, iron bioavailability was two to three-fold higher for the nori and sea lettuce samples, respectively (p≤0.05). In contrast, bioavailability from red ogo did not differ from spinach (Figure 2.1A).

Iron bioavailability was also converted to iron bioavailability per µg Fe in the well, (i.e. ng ferritin / mg cell protein/ µg iron), referred to as efficiency of iron bioavailability (Figure 2.1B). The efficiency of iron bioavailability was lowest for red ogo, highest for nori, and intermediate for spinach and sea lettuce. These results suggest that 1) for red ogo the low efficiency of iron uptake into the cells was a major factor limiting iron bioavailability from this seaweed, 2) that the high efficiency of iron uptake from nori was a major factor producing its relatively high iron bioavailability, and 3) that the high iron
content of sea lettuce (Table 2.7) in combination with intermediate efficiency of iron uptake combined to produce the high iron bioavailability from this seaweed.

Experiment 1 was repeated in three separate trials. All trials exhibited similar trends, but for brevity, only the results of Trial 2 were presented above. Bioavailability and efficiency data for all trials are shown in Figures 1-4 of Appendix.
Figure 2.1. A) Relative Fe bioavailability from seaweeds (nori, red ogo and sea lettuce) and spinach expressed as ng cell ferritin/mg cell protein produced in wells exposed to 1.5ml of digest containing 50 mg of dried sample (control samples shown in the insert). B) Relative efficiency of iron bioavailability from seaweeds and spinach expressed as ng cell ferritin/mg cell protein/ug Fe added to the wells. 0.5g dried sample was used in each digest. 1.5ml of digest was placed in the upper chamber and iron uptake by cells was assessed 18 h later by measuring cell ferritin formation. Bar values indicate means ± SD, n = 6 for food samples, n = 5 for blank and FeSO₄, and n = 2 for FeSO₄ + VitC. Data were log transformed prior to analysis to achieve equal variances. Bars with different letters are significantly different (p ≤ 0.05).
**Experiment 2: Rockweed, wakame & wawae’iole compared to spinach**

Experiment 2 utilized the same protocol as Experiment 1 but analyzed three different seaweeds: Rockweed, wakame and wawae’iole. All three seaweeds showed equal bioavailabilities to spinach (Figure 2.2A).

When comparing the relative efficiencies of iron absorption, all seaweeds except wawae’iole, showed a statistically significant decrease in efficiency compared to spinach (Figure 2.2B). This suggests that lower levels of efficiency contributed to low bioavailable responses in all seaweeds despite their having higher iron contents than spinach.
Figure 2.2 A) Relative bioavailability of seaweeds (rockweed, wakame and wawae’iole) and spinach expressed as ng cell ferritin/ mg cell protein produced in wells exposed to 1.5ml digest containing 50 mg of dried sample (controls shown in insert) B) Relative efficiency of iron bioavailability from seaweeds and spinach expressed as ng cell ferritin/mg cell protein/ug Fe added to the wells. 0.5g dried sample was used in each digest. 1.5ml of digest was placed in the upper chamber and iron uptake by cells was assessed 18 h later by measuring cell ferritin formation. Bar values indicate means ± SD, n = 6 for food samples, n = 5 for blank and FeSO₄, and n = 2 for FeSO₄ + VitC. Data were log transformed prior to analysis to achieve equal variances. Bars with different letters are significantly different (p ≤ 0.05).
**Experiment 3: Enhancement by vitamin C**

To provide information on the effects of a known enhancer of non-heme iron absorption on iron bioavailability from seaweeds, we tested the effects of adding Vitamin C to the seaweed digests. The addition of 0.28 mM Vit C significantly increased Fe bioavailability by 280%, 218% and 126% in spinach, nori, and sea lettuce, respectively (Figure 2.3). In contrast, Vitamin C did not enhance iron bioavailability in red ogo. The amount of Vit C used in this experiment (750 µg), when diluted in 15-ml sample digests, resulted in a Vit C concentration of 0.28 mmol/L, which approximated the physiological concentration of an oral intake of 100 mg Vit C diluted in 2 liters of stomach fluid.
**Figure 2.3.** Relative Fe bioavailability from seaweeds (red ogo, nori and sea lettuce) and spinach supplemented with vitamin C. Data are expressed as ng ferritin/ mg cell protein produced in wells exposed to 1.5ml of digest containing 50 mg of dried sample and added vitamin C (control samples shown in the insert). In each experiment, 0.5 mg dry sample was used in each 15 mL digest and 1.5 ml of digest was placed in the upper chamber. Iron uptake by cells was assessed 18 h later by measuring cell ferritin formation. Bar values indicate means ± SD, n = 6 for food samples, n = 5 for blank and FeSO₄, and n = 2 for FeSO₄ + VitC. Data were log transformed prior to analysis to achieve equal variances. Bars with asterisks are significantly different from the same sample without vitamin C added (* p ≤ 0.05; ** p ≤ 0.01)
Experiment 4: Evaluating the presence of inhibitors

Low bioavailability responses for high iron-containing seaweeds such as red ogo led us to believe that some seaweeds may contain a large amount of a potent inhibitor of iron absorption. To test this, we added 55 µg of iron (as FeSO₄) and 0.28 mM Vit C to each 15 mL seaweed digest. With additional iron as FeSO₄, there is an expected increase in bioavailable response. A significant increase in bioavailable iron as reflected by ferritin formation was seen in both nori and sea lettuce (178% and 202% increase, respectively) but not in red ogo. The lack of an increase in bioavailability responses in red ogo, even after the addition of extra iron, suggests the presence of a strong inhibitor of iron absorption in this seaweed, possibly Mn or polyphenols.
Figure 2.4. Relative Fe bioavailability from seaweed (red ogo, nori and sea lettuce) and spinach supplemented with Fe + Vit C. Data are expressed as ng ferritin/ mg cell protein produced in wells exposed to 1.5ml of digest containing 50 mg of dried sample with Fe and Vit C added (control samples shown in the insert). In each experiment, 0.5 mg dry sample was used in each 15 mL digest and 1.5ml of digest was placed in the upper chamber. Iron uptake by cells was assessed 18 h later by measuring cell ferritin formation. Bar values indicate means ± SD, n = 6 for food samples, n = 5 for blank and FeSO₄, and n = 2 for FeSO₄ + VitC. Data were log transformed prior to analysis to achieve equal variances. Bars with asterisks are significantly different from the same sample without vitamin C and iron added (* p ≤ 0.05; ** p ≤ 0.01).
2.4. DISCUSSION

The main objective of this study was to evaluate seaweeds as a source of iron by determining their iron content and iron bioavailability relative to spinach. Ten out of the 13 seaweeds samples analyzed had higher iron content per g/DM than spinach and most could be considered good or excellent sources of iron based on the iron content in a 10 gram (dry weight) serving. For example, it is possible to satisfy 100% of the Daily Value for iron by consuming about 5.2 g of dried dulse powder, 2.3 g of dried sea lettuce, or 6 g of dried nori (about one sheet). However, our bioavailability studies indicate that many of the high-iron containing seaweeds do not provide greater amounts of bioavailable iron per 10 g DM than spinach. Notable exceptions were nori and sea lettuce, which provided significantly more bioavailable iron than spinach and the other seaweeds tested. Additionally, dulse powder contains excessive amounts of the minerals Al, As, and Pb.

The levels of iron detected in our study generally fell within the ranges observed in previous reports on seaweeds. For example, other studies have shown an average iron content of about 500 and 1,000 µg Fe/g DW in nori (McDermid 2003; Garcia-Casal et al., 2007; Rodenas et al., 2009) and sea lettuce (Rizvi & Shameel 2001; Hou & Yan. 2008; Garcia-Casal et al., 2007), respectively. Our nori and sea lettuce samples contained an average iron content of 271 and 830 µg/g DW, respectively. However, it is important to note that the mineral contents of seaweeds, which are generally higher than those of terrestrial vegetables (Ruperez, 2002), vary between and within algal species. This variation may stem from differences in the environmental features of each region of harvest (water temperature, mineral content, pH, salinity, etc), which may affect mineral accumulation.
Many of the trace elements found in the algae are non-nutrients (Al, Cd, B, As, Ni, and Pb) but their content was generally below the toxic limits set by agencies such as the WHO. Exceptions to this were arsenic, which was found at toxic levels in hijiki, nori, rockweed and wakame, and aluminum in dulse powder. We did not measure iodine in this study, but some seaweeds can contain high levels of this mineral nutrient. Manganese was also notably high in our red ogo samples, 5.7 mg/10 gDM, which is greater than the tolerable upper intake level (UL) for children, and close to the UL for 9-13 year old females (6 mg/day). We do not know if high levels of manganese are common in red ogo, or if this was specific to our samples obtained locally in Honolulu.

There was a large variation in iron bioavailability among the seaweeds tested in this study. Red ogo, wakame, rockweed and wawae’iole all exhibited much lower bioavailability than nori and sea lettuce. The lower bioavailability could be due, in part, to the lower efficiency of iron bioavailability demonstrated in this study for red ogo, wakame, rockweed and wawae’iole.

We explored potential reasons for the variation in iron bioavailability among the seaweeds by adding Vitamin C and iron to some of the samples. Red ogo was unresponsive to the addition of vitamin C. Even with the addition of both extra iron and vitamin C, red ogo failed to show an increase in bioavailable iron which leads us to believe that there are inhibitory factors (possibly polyphenols or manganese) inherent in the seaweed itself that reduce the efficiency at which iron is absorbed into the mucosal cells. Polyphenols are thought to bind iron and prevent iron uptake by intestinal cells. Manganese inhibition of iron absorption has not been well studied, but it may act as a competitive inhibitor of iron transport.
into the enterocyte. The two minerals, which are both of a +2 charge, possibly compete for transporters.

The significantly higher bioavailability of nori and sea lettuce relative to the other seaweeds was due to either their higher iron content and/or their higher efficiencies of iron uptake. For example, nori contained relatively low amounts of iron compared to the other seaweeds, but it had the highest efficiency of iron bioavailability. This high efficiency resulted in nori providing more bioavailable iron per unit dry weight than any of the other samples tested, except for sea lettuce. The significant enhancement of iron bioavailability in nori by added vitamin C and iron implies the lack of strong inhibitors of iron absorption in this seaweed. This lack of intrinsic inhibitors may explain why nori had the highest efficiency of iron bioavailability among the seaweeds, and was also higher than spinach.

Sea lettuce was found to provide the greatest amount of bioavailable iron per unit dry weight among all the samples. This was due to it having the highest iron content in combination with the second highest efficiency of iron uptake. For example, sea lettuce contained three times as much iron as nori and exhibited about half the efficiency of iron bioavailability as nori. The net result is a greater amount of bioavailable iron provided by the sea lettuce sample. Sea lettuce also showed significant enhancement of iron bioavailability by added vitamin C and iron implying, as in nori, the lack of strong inhibitors of iron absorption in this seaweed. In addition, green algae in particular have been shown to be good, nonconventional sources of vitamin C, a known enhancer of iron absorption (Garcia-Casal et al., 2007). This inherent concentration of Vit C may have contributed to the available iron response seen by the bright green Ulva species.
To our knowledge, few studies have looked at iron bioavailability in seaweeds. One study by Moreda-Pineiro et al., (2012) measured percent dialysability in nori and sea lettuce samples. Dialysability and bioavailability encompass similar concepts but are not synonymous. Dialysability is defined as the fraction of a nutrient released from the food matrix into the gastrointestinal tract, and is thus available for intestinal absorption. However, the term bioavailability describes the proportion of a nutrient released from food that is absorbed by the intestine, and can therefore enter into the systemic circulation (Moredo-Pineiro et al., 2012). Results obtained for percent of relative dialysability (10% for sea lettuce and 20% for nori) do not match with our relative bioavailability values (about 55 ng ferritin for nori and 95 ng ferritin for sea lettuce). The lower percent dialysability in sea lettuce tells us that, the soluble Fe is still very absorbable, and supports the idea that high bioavailability in sea lettuce may be due to high iron content. The higher percent of soluble Fe in nori may explain the high efficiency observed in our study. In another study by Garcia-Casal et al. (2007), Radio-labeled iron added to the H2O in which rice was cooked in. This rice was then fed to human subjects with the addition of varying amounts of raw or cooked algae (Gracilaria, sea lettuce, and nori). However, this radio-labeled rice served as an extrinsic label to measure bioavailability from the rice itself and not the seaweed. But addition of seaweed to the rice meal enhanced extrinsic iron absorption from rice which points to a component in seaweed that may enhance iron absorption. Finally, in a study done by Shaw and Liu (2000), nori was shown to replete hemoglobin when included in the diets of iron-depleted rats. The authors suggested that this repletion rate makes nori comparable to other iron-fortified foods.
2.5. LIMITATIONS

The nutrient and mineral content of seaweeds is highly dependent on geographical origin and other ecological factors. Most of the seaweeds analyzed in this study were purchased commercially from local grocery stores and their exact origin is unknown. This variation makes it difficult to extrapolate species

The *in vitro* digestion/Caco-2 cell model is a commonly utilized, generally accepted method for estimating relative Fe bioavailability from foods. However, there are several limitations to this model that should to be addressed as they may reflect the interpretation of the results from this study.

First, although estimations of bioavailability in this system have been qualitatively validated against similar human trials regarding the effects of single food items and simple food interactions, in reality, the composition of the actual human diet is much more complex and the numerous possible combinations of foods are likely to influence the manner by which Fe intrinsic to the food is absorbed. Thus, estimates of bioavailability using the Caco-2 cell model may over or underestimate the magnitude by which Fe in foods is absorbed in the context of a whole diet.

Second, because estimations of bioavailability using this system are expressed on a relative (rather than absolute) basis, quantification of bioavailable iron as a percentage of total iron content is not possible.

Third, as with many *in vitro* studies, the accuracy of extrapolation to humans must be done with caution. The Caco-2 cell line is a human derived cell line, however, it is colonic in origin, and not from the duodenum of the small intestine; the segment where the majority of iron absorption occurs. Additionally, while Caco-2 cells have been shown to differentiate into
cells with similar morphological characteristics to that of the duodenum, Caco-2 cells lack the ability to produce mucus, which is thought to play a role in iron absorption because of its ability to stabilize soluble iron during the transitional increase in luminal pH. Therefore, in the absence of mucus, values of bioavailability derived using only Caco-2 cells may be over- or under-estimated.

Lastly, because of the configuration of the Caco-2 cell model (in which cell monolayers are grown on the bottom of 6-well plates), the system is limited to assessing bioavailability of foods at the point of entrance into the mucosal cell. In humans however, absorption also involves intracellular movement along with export into circulation. Thus, any affect of inhibition and/or enhancement of bioavailability occurring at either of these points of absorption in the mucosal cell would not be detected in the Caco-2 cell system.

2.6. FUTURE STUDIES

Measures of bioavailability may be affected by the presence of various inhibitors and enhancers in the seaweed matrix. Further research is needed to assess the levels and significance of these inhibitors and enhancers (i.e. polyphenols, manganese, dietary fiber, vitamin C, etc.). In addition, further research is needed to assess the effects of ecological variations (e.g. season of harvest, water quality, etc.) on mineral content and bioavailability in seaweeds. Also as previously mentioned, seaweeds have the ability to bio-accumulate other minerals and heavy metals from their surrounding marine environment. Thus, if seaweeds are to be recommended as a food source of iron, it would be important to examine the concentration and bioavailability of minerals present that could pose risk for toxicity.
2.7. CONCLUSIONS

Seaweeds and other marine algae have been studied as an alternative source of nutrients, such as iron. However, because high iron content does not necessarily imply high iron utilization, the objective of our study was to determine both the mineral composition of algae collected commercially and locally in Hawaii, and evaluate iron bioavailability from these algae using an *in vitro* digestion/Caco-2 cell model.

Our results present new information on the mineral content of common seaweeds and seaweeds consumed in Hawai‘i. Furthermore, data from this study may be able to provide additional data to the National Nutrient Database for Standard Reference. Currently, the mineral content of many of the seaweeds analyzed in this study is not among the foods listed for nutrient content in the National Nutrient Database. Raw kelp, nori and wakame are listed, but the majority of minerals listed are incomplete with only one data point or the data is imputed. In addition, the database does not include nutritional information on the seaweeds in dried form. Processing and drying methods become particularly important in regards to seaweed because most are not eaten fresh. The present investigation into the mineral content of seaweeds will provide important nutrient information for consumers. This information could be used to improve the nutritional quality of the diet, in particular plant-based diets.

According to the results presented in this study, many seaweeds may not be highly efficient bioavailable sources of iron. Only nori and sea lettuce were found to provide significantly more bioavailable iron than spinach. Although many seaweeds are rich in iron content, they may not necessarily provide bioavailable iron to the diet. Moreover, the relatively high content of arsenic and other toxic minerals found in many high-Fe seaweeds may prove detrimental to individuals who consume large amounts. It is important to evaluate
other algae species, especially those growing uncontrollably in many tropical coasts. The use of algae shown to contain bioavailable iron, with appropriate nutritional education, could help solve ecological and iron deficiency problems in some underdeveloped and developed countries.
LITERATURE CITED


APPENDIX

Table 1. Iron content and grams of food in digests applied to Caco-2 cells in bioavailability experiments

<table>
<thead>
<tr>
<th>Sample</th>
<th>grams of DM per digest</th>
<th>μg Fe per well</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Seaweeds</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Red Ogo</td>
<td>0.5</td>
<td>25.95</td>
</tr>
<tr>
<td>Nori</td>
<td>0.5</td>
<td>13.54</td>
</tr>
<tr>
<td>Sea lettuce</td>
<td>0.5</td>
<td>41.51</td>
</tr>
<tr>
<td>Wakame</td>
<td>0.5</td>
<td>19.11</td>
</tr>
<tr>
<td>Wawae'iole</td>
<td>0.5</td>
<td>10.71</td>
</tr>
<tr>
<td>Rockweed</td>
<td>0.5</td>
<td>35.20</td>
</tr>
<tr>
<td><strong>Spinach</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spinach (cooked)</td>
<td>0.5</td>
<td>8.30</td>
</tr>
<tr>
<td><strong>Control</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ferrous sulfate</td>
<td>0</td>
<td>5.5</td>
</tr>
</tbody>
</table>
Table 2. Relative iron bioavailability using 0.5 grams dry matter in each digest\textsuperscript{1}

<table>
<thead>
<tr>
<th>Sample</th>
<th>Relative iron bioavailability per 0.5 g DM</th>
<th>Relative efficiency of iron absorption per 0.5 g DM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ng ferritin / mg cell protein / ug Fe</td>
<td>ng ferritin/ mg cell protein/ 0.5g DM</td>
</tr>
<tr>
<td><strong>Seaweeds</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Red Ogo</td>
<td>33.80 ± 7.88</td>
<td>0.43 ± 0.10</td>
</tr>
<tr>
<td>Nori</td>
<td>18.59 ± 5.41</td>
<td>1.37 ± 0.40</td>
</tr>
<tr>
<td>Sea lettuce</td>
<td>37.99 ± 1.34</td>
<td>0.93 ± 0.03</td>
</tr>
<tr>
<td>Wakame</td>
<td>15.36 ± 4.44</td>
<td>0.80 ± 0.23</td>
</tr>
<tr>
<td>Wawae'iole</td>
<td>13.22 ± 3.88</td>
<td>0.38 ± 0.11</td>
</tr>
<tr>
<td>Rockweed</td>
<td>17.84 ± 1.95</td>
<td>1.70 ± 0.18</td>
</tr>
<tr>
<td><strong>Spinach</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spinach (cooked)</td>
<td>14.12 ± 1.31</td>
<td>1.70 ± 0.16</td>
</tr>
<tr>
<td><strong>Quality Controls</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blank</td>
<td>12.27 ± 1.07</td>
<td>--</td>
</tr>
<tr>
<td>Ferrous sulfate</td>
<td>19.79 ± 7.91</td>
<td>3.60 ± 1.44</td>
</tr>
<tr>
<td>Ferrous sulfate plus Vit C</td>
<td>123.06 ± 32.35</td>
<td>22.37 ± 5.88</td>
</tr>
</tbody>
</table>

\textsuperscript{1}Values are means ± SD (n = 6, n = 5 for Sea lettuce, blank, and ferrous sulfate, n= 2 for ferrous sulfate + Vit C); means for Wakame, wawae'iole and rockweed taken from Experiment 2. All other values taken from Trial 2 of Experiment 1.
A. Experiment 1 (Red Ogo, Sea Lettuce, Nori): Trial 1

**Figure 2.** A) Relative Fe bioavailability from seaweed (nori, red ogo and sea lettuce) and spinach expressed as ng cell ferritin/ mg cell protein produced in wells exposed to 1.5ml digest containing 50 mg of dried sample (control samples as an insert). B) Relative efficiency of iron absorption from seaweed samples and spinach expressed per ug Fe. 0.5g dried sample was used in each digest. 1.5ml of digest was placed in the upper chamber and iron uptake by cells was assessed 18 h later by measuring cell ferritin formation. Bar values indicate ± SD, n = 6, n = 5 for blank and FeSO₄, and n = 2 for FeSO₄ + VitC. Data was log transformed prior to analysis to achieve equal variances. Bars with different letters are significantly different (p ≤ 0.05).
B. Experiment 1 (Red Ogo, Sea Lettuce, Nori): Trial 3

A) Relative bioavailability of seaweeds

![Bar chart showing relative bioavailability of seaweeds](image)

Food Samples

B) Relative efficiency of iron bioavailability from seaweeds

![Bar chart showing relative efficiency of iron bioavailability](image)

**Figure 3.** A) Relative Fe bioavailability from seaweed (nori, red ogo and sea lettuce) and spinach expressed as ng cell ferritin/ mg cell protein produced in wells exposed to 1.5ml digest containing 50 mg of dried sample (control samples as an insert). B) Relative efficiency of iron absorption from seaweed samples and spinach expressed per ug Fe. 0.5g dried sample was used in each digest. 1.5ml of digest was placed in the upper chamber and iron uptake by cells was assessed 18 h later by measuring cell ferritin formation. Bar values indicate ± SD, n = 6, n = 5 for blank and FeSO₄, and n = 2 for FeSO₄ + VitC. Data was log transformed prior to analysis to achieve equal variances. Bars with different letters are significantly different (p ≤ 0.05).