NUTRITIONAL COMPOSITION OF TWO EDIBLE HAWAIIAN SEAWEEDS: *AHNFELTIOPSIS CONCINNA* AND *GRACILARIA SALICORNIA*

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INTRODUCTION

Since ancient times humans have used seaweeds as a source of nourishment. Many people from different countries around the world, including Japan, India, South America, Africa, Australia, South Eastern Asia, Micronesia, North America, and particularly the State of Hawaii, include a variety of seaweeds in their daily food intake. Some of the nutritional elements essential to humans can be found in seaweed. The three essentials elements that sustain the human body are carbohydrates, lipids and proteins. The nutritional elements that are found in seaweeds and can be quantitatively measured. The measurement of these essential nutritional elements, plus the quantitative study of ash, and minerals is the subject of this paper.

The diet of the early cultures of the Hawaiian Islands included the consumption of seaweed. Today there are many types of seaweeds consumed in Hawaii. *Limu*, (Hawaiian for seaweed) has been eaten by Hawaiians for hundreds of years. It is usually eaten with raw fish (poke) and poi (Abbott 1996). This ancient meal was and still is a balanced meal consisting of proteins, carbohydrate, lipids and minerals. Today *limu* is a commodity and can be found in leading supermarkets all throughout Hawaii. It is surprising that for such a popular food item in the Hawaiian Islands, there is almost no information on the nutritional values of Hawaiian *limu*.

Studies relating to the nutritional value and the composition of seaweeds have been undertaken in many regions of the world, but no studies have been completed in the Hawaiian Islands. Such a study performed in India documents the biochemical composition of five species of seaweeds (Naidu et al. 1993). Another study done, in Puget Sound, Washington, analyzed sixteen species of seaweed for the content of

vitamins (Norris et al, 1937). Other studies from around the world include the Philippines. In this study scientists analyzed the nutritive value of a few seaweeds, including the proximate, amino acid and vitamin composition (Portugal et al. 1983). In Yucatan, Mexico a study about the chemical and mineral composition of edible seaweeds was performed. Another study in Florida analyzed the proximate constituents and lipid chemistry in two species of *Sargassum* (Hamdy and Dawes 1988). These studies are good references that provide quantitative data that can be used as comparisons to this study. These papers do not give the nutritional values of Hawaiian species. From the existence of this void arose the necessity to do my own analysis and experiments on edible indigenous Hawaiian species.

Most consumers would like to know the nutritional composition of what they are eating. Upon completion of this project I found out what seaweeds were high in protein and minerals, and what seaweeds were low in lipids and carbohydrates. I analyzed two types of popular edible Hawaiian seaweeds, *Gracilaria salicornia* and *Ahnfeltiopsis cocinna*. The samples were collected from different sites on the Big Island of Hawaii, as well as on the island of Oahu. Cabbage (*Brassica oleracea)* was also analyzed, as a control and comparison to a vegetable most people are familiar with. After obtaining the values for protein, lipid, carbohydrate, ash, and mineral content I compared each species at the different sites. I hypothesized that the nutritional value of the analyzed species will not vary among sites within the same species, nor will the nutritional value vary between different species.

As stated above, this study found out which Hawaiian seaweeds were high in protein and minerals, and which seaweeds were low in lipids and carbohydrates. This

important information can eventually be applied in the use of the Hawaiian seaweed as a source of nutrition, whether the seaweeds are eaten in their natural state or processed for the extraction of nutrients that can also be used as food supplements. In order to better understand the importance of the various analyzed, it is essential to review what these components are and what their nutritional function is.

Carbohydrates are chemical compounds composed of carbon, hydrogen and oxygen. In cellular metabolism, carbohydrates provide chemical energy and material for the construction of more complex molecules (West 1961). Carbohydrates are the most abundant of all organic substances, more than all other organic substances combined grouped together. The organic structure of all plants and animals is primarily composed of carbohydrates. Cellulose is the most common carbohydrate, found in wood and other fibers of plants, such as seaweed. Carbohydrates are essential to sustain the human body. For humans, the main sources of carbohydrate are starches, found in grains, roots and tubers. Starches are metabolized into glucose, parts of this glucose are converted into fats, and some react with other nitrogen containing compounds to become amino acids.

Carbohydrates are classified into three different groups:

1. *Monosaccharides* (simple sugars), a monosacharide cannot be hydrolyzed to a simpler compound. Example: Glucose (nature contains more glucose units than any other organic group). 2. *Disaccharides* (double sugars) these are carbohydrates that are made up of two monosaccharides. Upon hydrolysis a molecule of disaccharides yields two molecules of monosaccharides. Examples: malt sugar (maltose), milk sugar (lactose) and sucrose (cane or beet sugar) .3. *Polysaccharides* (complex

sugars) compounds composed of hundreds or even thousands of monosaccharides units per molecule. As is in the case of disaccharides, these units are held together by glycoside linkages, which can be broken by hydrolysis in the respective resulting monosaccharides. Examples: starch and cellulose, both produced in plants from carbon dioxide and water and sunlight (West 1961). For the purpose of this experiment specifically, the soluble carbohydrate content was analyzed and calculated.

Lipids belong to a group of naturally occurring substances that are not soluble in water, but soluble in solvents, such as ether, chloroform, alcohol and benzene. Lipids of biological origin are a mixture of a variety of classes of substances. These classes represent a number of molecular shapes and sizes, for example simple, short chained fatty acids versus very large and complex molecules (Handler 1961). Because of the complexity of the nature of lipids, the determination of their molecular structure and functions is complex. Lipids have a great biochemical importance, as they are the main storage form of energy. They are used commercially to make soap and detergent.

Lipids may be defined in three ways: (1) by their solubility in organic solvents (such as ether, chloroform, alcohol and benzene), (2) by the presence of fatty acids, (3) by how living organisms utilize them. For the purpose of this study, the solubility method was used for crude lipid extraction.

Proteins may be defined as complex nitrogen containing organic compounds. Like, lipids and carbohydrates, proteins contain carbon, hydrogen and oxygen, but in addition, they always contain nitrogen and usually also sulfur. They are found in all

plant and animal cells, making up a large part of the protoplasm of the cell. The word protein comes from the Greek word *proteios,* which means primary (Hickman 1961). Without proteins, life is not possible on earth. The function of protein in human diet is not primarily to supply energy, as is in the case of carbohydrates and lipids, but to provide building blocks (amino acids) for the living tissue of the organism. Proteins are characteristic of their particular plant or animal origin. The analyses of proteins falls into four categories: (1) by the complete breakdown of the protein into amino acids, (2) by the partial breakdown to obtain dipeptides, tripeptides, or varied polypeptides (peptides are chains of amino acids), (3) by systematically removing one amino acid after the other from one end of the protein chain, (4) The study of the intact molecule by such means as: physical-chemistry, colloid- chemistry, immuno-chemistry and crystallography (West 1966). This experiment extracted crude carbohydrate content.

MATERIAL AND METHODS

Collection of Samples

Samples of *Ahnfeltiopsis cocinna* were collected from the Big Island at two sites, Onekahakaha, Hilo and at the old airport in Kona. *Gracilaria salicornia* was collected from Onekahakaha, Hilo and obtained from Royal Hawaiian Sea Farms in Kona on the Big Island and from Heeia Fish Ponds and Kaneohe Bay on Oahu. Cabbage (*Brassica oleracea*) was purchased from KTA supermarket.

Drying Procedure

All the tests require that the seaweeds be in powder form. Collection and drying is the same for all species and tests. Approximately 1 kilogram of healthy, clean seaweed was collected in food grade plastic bags and transported to the lab in a cooler. The sample was then rinsed and cleaned with filtered seawater to remove all the epiphytes, sand debris and small organisms. A voucher specimen was selected to press for the herbarium. The plant material was spun for 30 seconds in a salad spinner. Then the samples were weighed (wet weight) and put on foil trays. The trays were then placed in a 60-degree Celsius oven for at least 24 hours. They were removed from the oven when they were at a constant weight. The weight was recorded, and the percent water of the sample was calculated (wet weight – dry weight = weight of water). The seaweed was ground in an electric coffee grinder until a fine powder was formed. The powder was placed in a labeled glass jar and stored in the refrigerator until further use.

Ashing Method

To determine percent ash 25-100 milligrams of seaweed powder was weighed out into clean, dried, calibrated crucibles. The weight of the powder was recorded. The covered crucibles were then placed in a gravity oven at 60-degrees Celsius for 10-20 minutes, to warm the samples. After this, the crucibles were transported to the muffler furnace set at 500 degrees Celsius for 4 hours (Carefoot, 1985). The lids were lifted about 2 hours later so that gases could be released. After 4 hours the covered crucibles were removed and cooled for one hour on an asbestos pad, then placed they were placed in a desiccator until they reached room temperature, about one hour.

The crucibles were reweighed with lids on and powder remembering, always to use gloves (because our hands have oils which might add to the weight of the crucible). After the weight was recorded, percent ash was calculate using these formulas:

Ash = Final weight of crucible with powder-crucible

Ash free dry weight = Initial weight of powder-ash

% Ash relative to total dry weight = Ash/Initial weight of powder x (100)

The weight loss by ashing consists of water and biochemicals. The intense 500-degree Celsius temperature exposure turns these contents into water and carbon dioxide. Ashing gets rid if all the organic compounds and only the inorganic matter is left, leaving us with the percent ash content.

Analysis of total lipid content

A gravimetric method was used to determine total lipid content (Dawes 1998). To test for percent lipid content 100 milligrams of seaweed powder was placed in a vial, and then the vial was filled with 25 milliliters of a chloroform-methanol mixture. The vials were then capped and heated in a 60-degree Celsius water bath for 30 minutes. After the 30 minutes the sample was cooled and refilled with the C:M mixture. The cap was tightened and the vial was shaken for 3 minutes. Next, the solution was filtered through a Millipore filter set-up with Whatman No. 541 filter paper. 4-ml of distilled water was added to the vial, capped and shaken for 5 minutes. After centrifuging for five minutes the upper aqueous layer was removed using a glass pipette. The solvent was then evaporated at 60-degrees under a filtered cotton-filled Nitrogen gas stream. While waiting for the solvent to evaporate glass shell vials were weighed. The lipid extract was transferred to the glass shell vial. The solvent was

evaporated again and the vial was reweighed. This formula was used to find the percent lipid content:

% Lipid = (lipid weight x 3/2)/ mg of dried seaweed x (100)

Analysis of protein

To determine percent protein the Lowry method was used because it is the most common, therefore the values can be compared to other studies (Lowry et al. 1951). 10 milligrams of seaweed powder were placed in a 15 mL test tube. Exactly 5.0 mL of 1 N NaOH were added to the test tube, covered with Para film and let sit for 12 hours. After letting it sit, the solution was centrifuged for 5 minutes at 500 rpm. 0.5 mL of the extract were pipetted into a clean 10 mL test tube. 5.0 mL of alkaline copper citrate reagent was added to the test tube. It was mixed immediately after the addition to avoid precipitation. The alkaline copper citrate reagent must be freshly made each day in the following sequence or a precipitate will form: 2 mL of 2% $CuSO_4 \times 5\ H_2O$ to 2 mL of 4% sodium citrate to 96 mL of 3% Na_2CO_3 in 0.1N NaOH. The solution was mixed in a vortex mixer and it stood for 10 minutes. Exactly 0.5 mL of the 1 N Folin-Ciocalteu phenol reagent was added, mixed well immediately and allowed to stand for 30 minutes. The mixture was decanted into a cuvette and the absorption was read at 660 nm in a spectro-photometer. The same cuvette was used for all replicates and in between each replicate it was rinsed 3 times with double-distilled water. The mg protein for 0.5 mL of solution was determined by comparing the absorption of the sample with the standard curve using 0.00- to 0.25-mg concentrations of bovine serum albumin in 1 N NaOH.

The percent protein is calculated using the following equation:

$$\textbf{\% Protein} = \frac{\text{mg protein} \times 10}{\text{mg plant tissue}} \times 100\%$$

Analysis of soluble carbohydrates

To test for soluble carbohydrates, a colorimetric procedure was followed (Dawes 1998). 5 mg of the seaweed powder was placed in a 15-mL centrifuge tube and 10mL of 5% trichloroacetic acid (TCA) was added. The tube was marked at the level at which this solution began in order to replenish it with distilled water after heating. The mixture stood over night. The tubes were heated in a hot-water bath for 3 hours at 80 to 90 degrees C. The tubes were gently shaken at least 3 times during the 3 hours to allow adequate extraction. After the water bath the solution cooled. Distilled water was added to return levels to initial mark. The tubes were centrifuged for 5 minutes. Exactly 0.2 mL of the solution was pipetted from below the surface (to avoid scum) into a 10-mL test tube. 1 mL of 5% phenol was added and mixed in a vortex mixer for 10 seconds. Rapidly 5 mL of concentrated reagent grade H_2SO_4 was added and carefully mixed (e.g., Vortex mixer for 10 sec). BE CAREFUL this reaction is exothermic. The tubes cooled for 30 min before the absorption was measured at 490 nm in a cuvette (The same cuvette was used for all the replicates and in between each replicate it was rinsed 3 times with DDW). When the cuvette is placed in the spectrophotometer latex gloves were worn and the cuvette was cleaned with kimwipes. The cuvette needed to be placed at the same spot in the spectrophotometer each time. The percent carbohydrate was calculated based on absorptions taken from a standard curve of 0.00 to 0.25 mg glycogen.

The percent soluble carbohydrate was calculated using the following equation:

$$\% \text{ Carbohydrate} = \frac{\text{mg carbohydrate}}{\text{mg plant tissue}} \times 100$$

Mineral Analysis

Samples of *Ahnfeltiopsis concinna* and *Gracilaria salicornia* were set to Waters Agricultural Laboratories Inc. in Camilla, Georgia for mineral analyses.

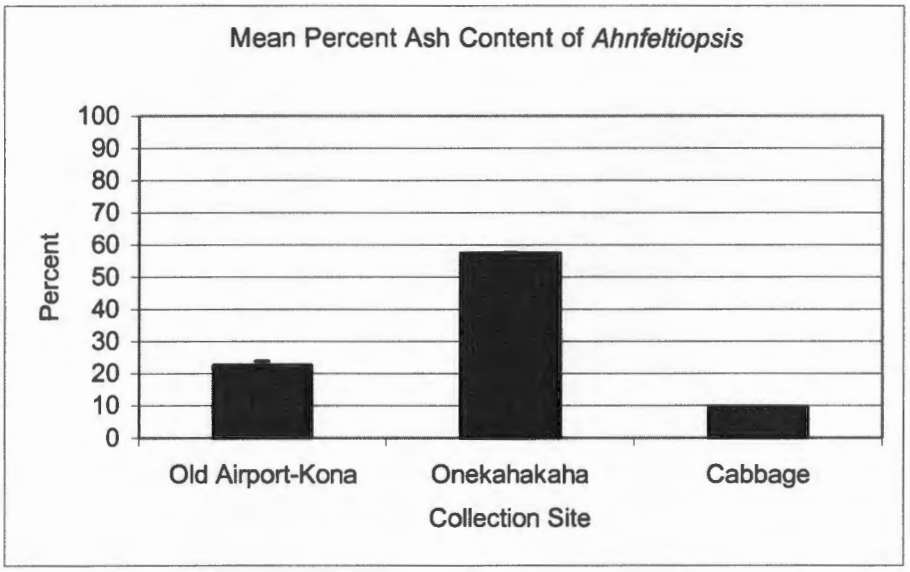
RESULTS

Table 1: Nutritional values of *A. concinna* and *G. salicornia*

Species	Collection Site	Ash (%)	Lipid (%)	Protein (%)	Carbohydrate (%)
A. concinna	Old Airport Kona	22.7	1.5	5.4	33.4
A. concinna	Onekahakaha Hilo	57.5	2.2	5.6	31.9
G. salicornia	Onekahakaha Hilo	52.1	1.6	5.3	20.0
G. salicornia	Royal Hawaiian Sea Farms	57.6	2.2	11.3	14.3
G. salicornia	Heeia Fish Ponds Oahu	61.3	1.9	4.4	18.5
G. salicornia	Kaneohe Bay Oahu	45.5	1.5	4.0	24.6
Cabbage	KTA	9.5	4.3	14.2	45.1

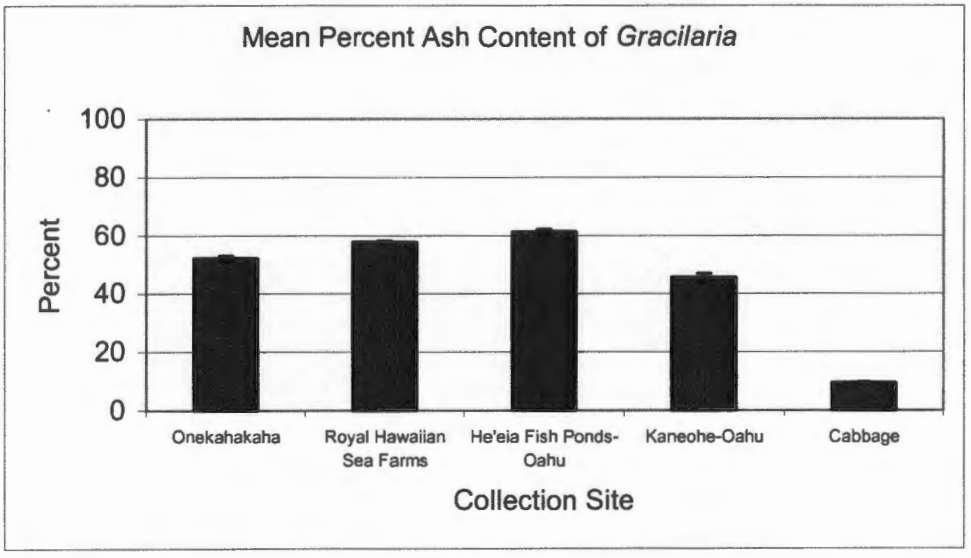
Table 2: Mineral values for *A. concinna* and *G. salicornia*

Mineral/Element	***A. concinna***	***G. salicornia***
Nitrogen (%)	1.46	0.9107825
Phosphorus (%)	0.1035	0.18175
Potassium (%)	3.003	20.40625
Magnesium (%)	0.814	0.455
Calcium (%)	0.463	0.57825
Sulfur (%)	7.777	3.878
Boron (ppm)	314.971	398.7705
Zinc (ppm)	15.7475	10.91975
Manganese (ppm)	43.841	82.3975
Iron (ppm)	78.8825	224.86875
Copper (ppm)	3.051	3.56025

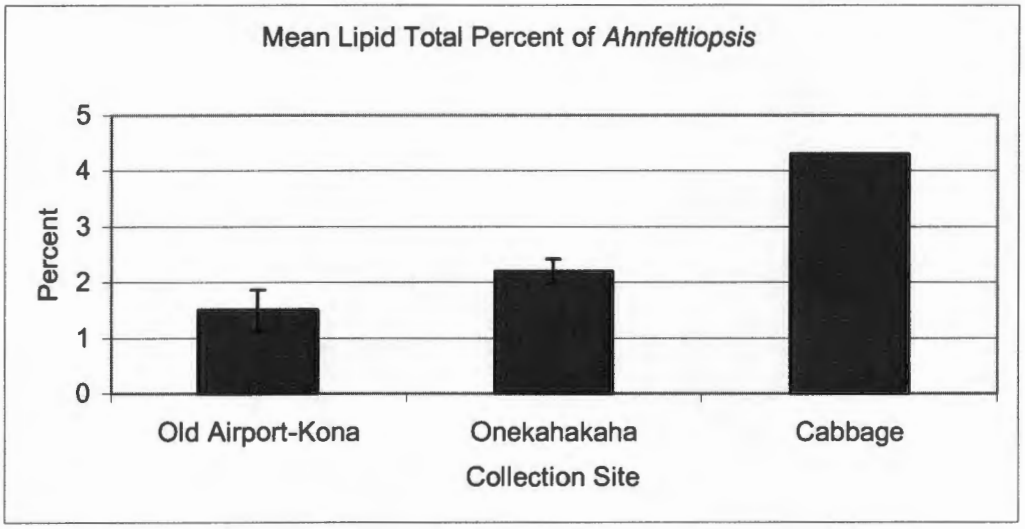


Graph 1

Graphs 1 and 2 illustrate the mean ash content of *A. cocinna* and *G. salicornia*, respectively. Cabbage is displayed on the right for comparison.

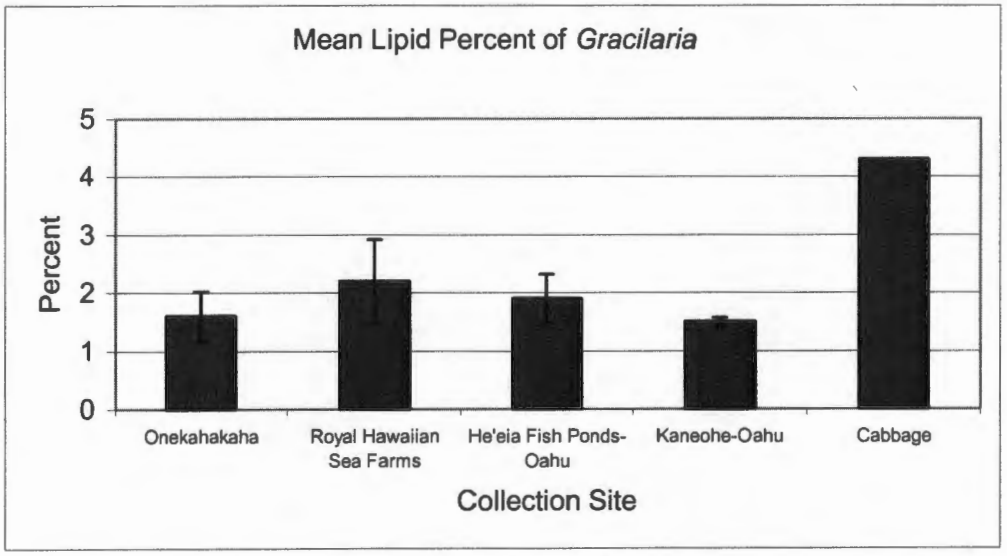


Graph 2

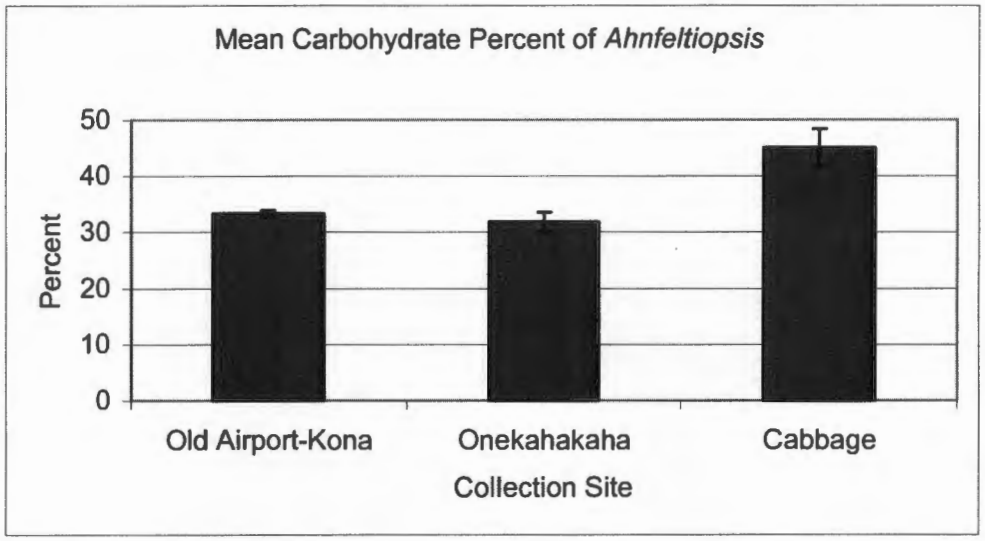


Graph 3

Graphs 3 and 4 display the mean lipid percent of *A. concinna* and of *G. salicornia*, respectively. Cabbage is displayed on the right for comparison.

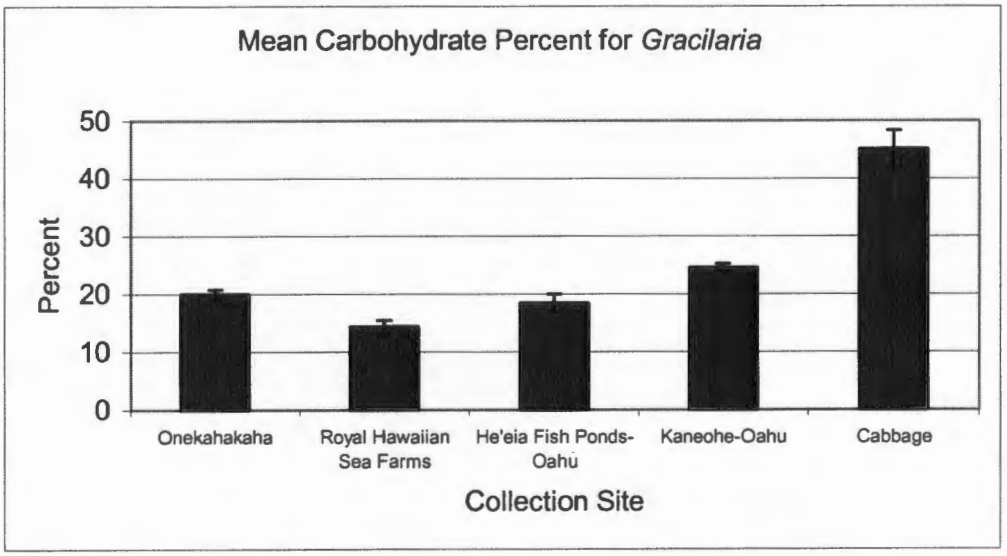


Graph 4

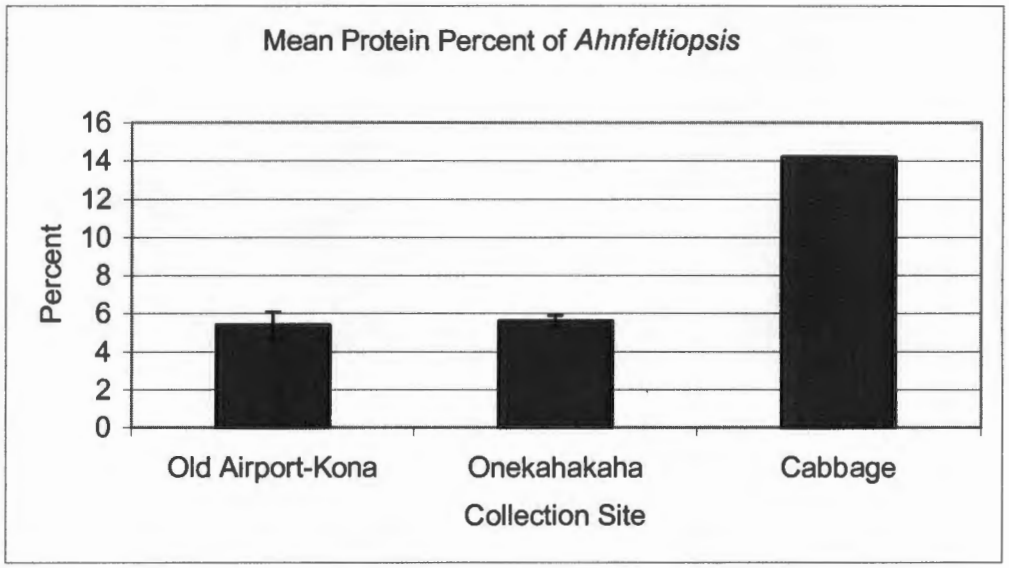


Graph 5

Graphs 5 and 6 display the mean carbohydrate percent of *A. concinna* and of *G. salicornia*, respectively. Cabbage is displayed on the right for comparison.

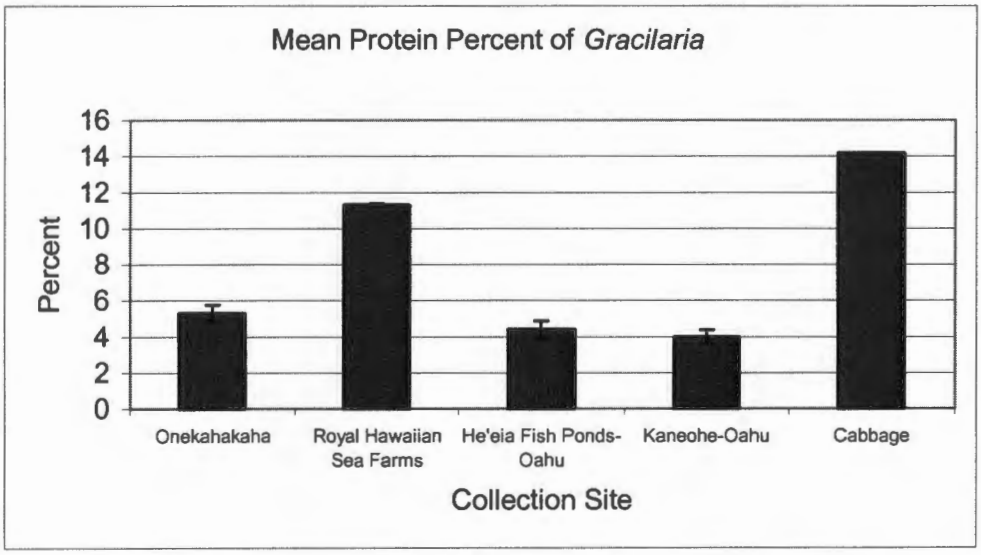


Graph 6

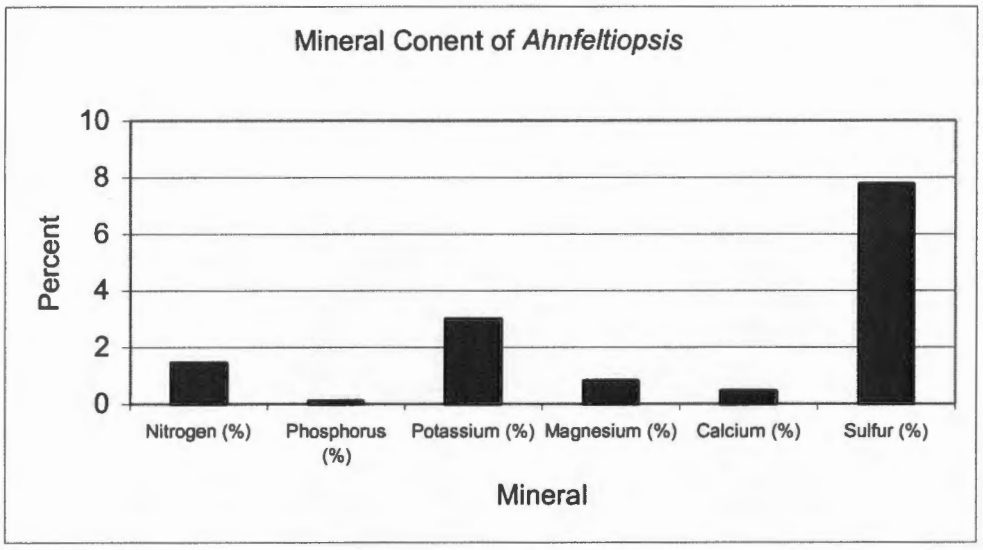


Graph 7

Graphs 7 and 8 display the mean protein percent of *A. concinna* and of *G. salicornia*, respectively. Cabbage is displayed on the right for comparison.

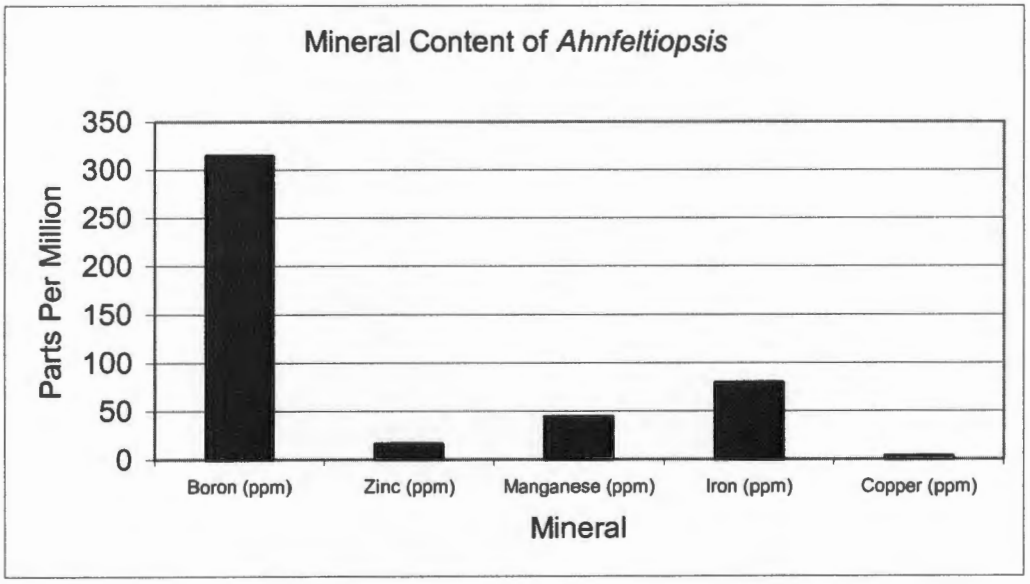


Graph 8

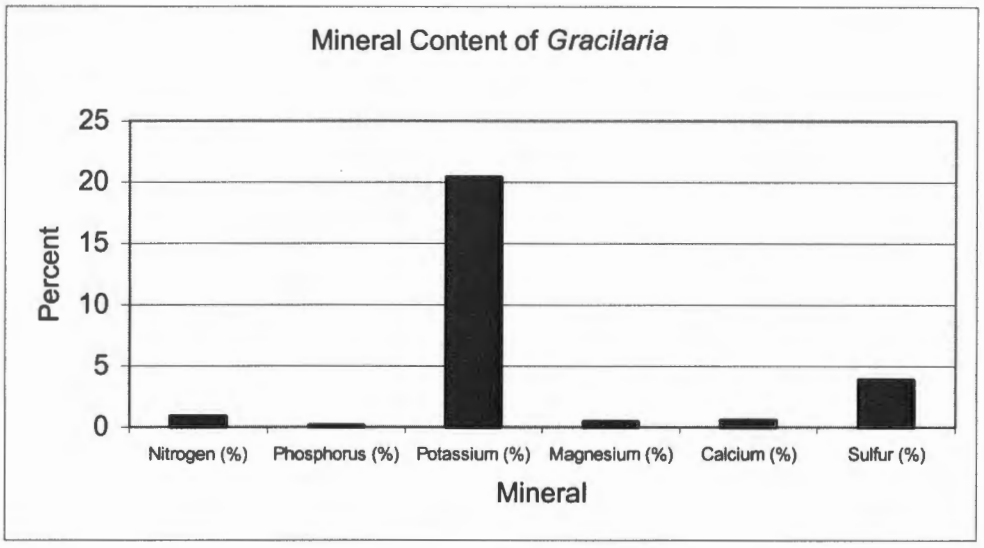


Graph 9

Graph 9 displays the minerals found in *A. concinna* in percent. Graph 10 displays the minerals found in *A. concinna* in parts per million (ppm).

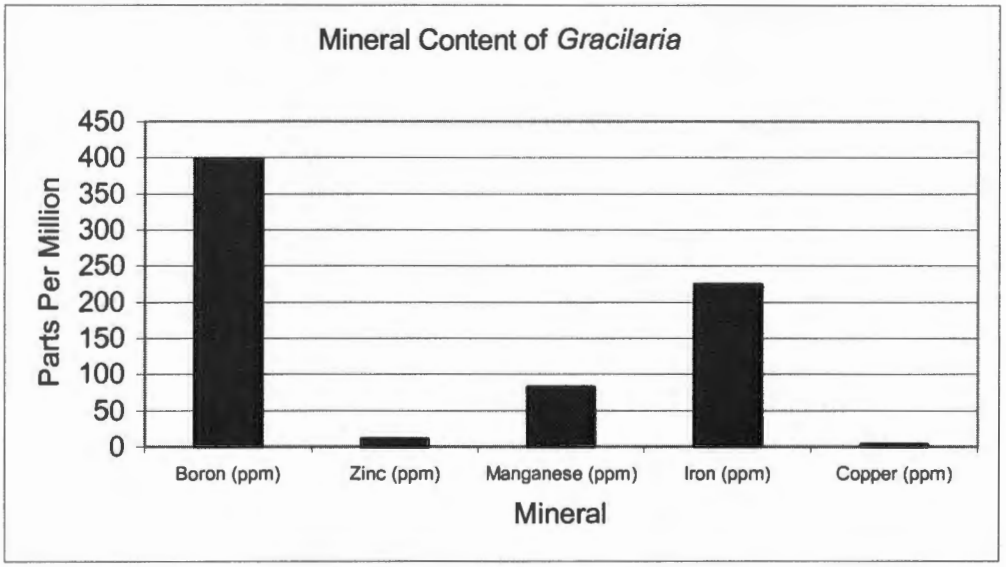


Graph 10



Graph 11

Graph 11 displays the minerals found in *G. salicornia* in percent. Graph 12 displays the minerals found in *G. salicornia* in parts per million (ppm).



Graph 12

DISCUSSION

The ash content is an indicator of the amount of inorganic matter (Portugal et al. 1983). The ash content (Table 1) of *A. concinna* ranges from 22.7-57.5%. In *G. salicornia* the ash content ranges from 45.5-61.3%. The ash content in *G. salicornia* is fairly high compared to other species of *Gracilaria*. The ash content of *G. changgi* is 22.7% (Norziah and Ching 2000) and *G. coronipifolia* is 20.9% (Portugal et al. 1983). Both *A. concinna* and *G. salicornia* have higher ash content than that of cabbage, which is 9.5%.

The lipid content for the majority of seaweeds is between 1% and 3 % (Norziah and Ching 2000). The lipid content (Table 1) ranges from 1.5-2.2% in both *A. concinna* *G. salicornia*. The lipid content of *G. coronipifolia* is 0.8%, less than *G. salicornia* (Norziah and Ching 2000). *A. concinna* and *G. salicornia* lipid contents are less than cabbage at 4.3%. According to Norziah and Ching (2000) a variety of vegetables, such as carrots, broccoli and lettuce have lipid content less than 1.0%, which is somewhat comparable to seaweeds.

The protein content (Table 1) of *A. concinna* has a small range of 5.4-5.6%. *G. salicornia* ranges from 4.0-11.3%. The protein content of the majority of *Gracilaria* species is between 7 and 13% (Norziah and Ching 2000). *G. changgi* has 6.9% protein (Norziah and Ching 2000), and *G. coronipifolia* has 9.8% protein (Portugal et al. 1983). Cabbage has 14.2% protein content, lower than both *A. concinna* and *G. salicornia*. The high value of 11.3% in *G. salicornia* was obtained from the species at Royal Hawaiian Sea Farms. This high value could be due to the fact that this sample was cultured and not grown in the wild.

The carbohydrate content (Table 1) of *A. concinna* ranges from 31.9-33.4. *G. salicornia* has a range of 14.3-24.6%. *G. coronipifolia* has a higher carbohydrate content of 41.8% (Portugal et al. 1983). Both *A. concinna* and *G. salicornia* have less carbohydrate content than cabbage, which has 45.1% carbohydrate content.

The minerals (Table 2) analyzed were nitrogen, phosphorus, potassium, magnesium, calcium, sulfur, boron, zinc, manganese, iron and copper. The first six mentioned were measured in percent (%), while the latter were found in such trace amounts that they were recorded in ppm, parts per million equals 0.0001%. As seen in table 9, *Ahnfeltiopsis concinna* contains a surprisingly large amount of sulfur and potassium, 7.7% and 3.0% respectively. Table 10 displays high relatively large amount of boron, manganese and iron found in *Ahnfeltiopsis concinna*. Table 11 make it obvious that potassium is the main mineral found in *Gracilaria salicornia*. Like *A. concinna, G. salicornia* has a relatively large amount of boron, manganese and iron. In both graph 10 and 12 zinc and copper are present in such minuscule amounts it is as though these minerals almost don't exist.

This study has concluded that the nutritional value does in fact vary among sites within the same species, and the nutritional value does vary between different species.

CONCLUSION

As determined, the same species, whether it is *Ahnfeltiopsis concinna* or *Gracilaria salicornia,* has varying nutritional values from site to site. There are a variety of factors that might contribute to the variation in the nutritional values of these two species of red algae. Such factors might include, nutrient contents of ambient

seawater, genetic differences and temporal variations, such as water temperature, salinity and tidal regime. This study set out to analyze the nutritional composition of two edible Hawaiian seaweeds. With this accomplished the void has been filled for two more edible Hawaiian seaweeds. This project found out what seaweeds were high in protein and minerals (*G. salicornia* from Royal Hawaiian Sea Farms), and what seaweeds were low in lipids (*A. concinna* from the old airport in Kona and *G. salicornia* from Onekahakaha in Hilo) and carbohydrates (*G. salicornia* from Royal Hawaiian Sea Farms). Future work relating to this study might include researching trends, such as, seasonal variation, red vs. greens vs. browns and cultured species vs. the same wild species.

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