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REFRIGERATED SHELF LIFE EVALUATION AND EFFECTS OF MINIMAL PROCESSING ON ANTIOXIDANT CAPACITY OF FRESH SEA VEGETABLES FROM NEW ENGLAND

By

Dhriti Nayyar

B.Sc. North Carolina State University, 2012

A THESIS

Submitted in Partial Fulfillment of the

Requirements for the Degree of

Master of Science

(in Food Science and Human Nutrition)

The Graduate School

The University of Maine

August 2016

Advisory Committee:

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THESIS ACCEPTANCE STATEMENT

On behalf of the Graduate Committee for Dhriti Nayyar, I affirm that this manuscript is the final and accepted thesis. Signatures of all committee members are on file with the Graduate School at the University of Maine, 42 Stodder Hall, Orono, Maine.

August 19, 2016

Dr. Denise Skonberg, Associate Professor of Food Science and Human Nutrition

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REFRIGERATED SHELF LIFE EVALUATION AND EFFECTS OF MINIMAL PROCESSING ON ANTIOXIDANT CAPACITY OF FRESH SEA VEGETABLES FROM NEW ENGLAND

By Dhriti Nayyar

Thesis Advisor: Dr. Denise Skonberg

An Abstract of the Thesis Presented
in Partial Fulfillment of the Requirements for the
Degree of Master of Science
(in Food Science and Human Nutrition)
August 2016

Sea vegetables (also known as seaweeds) are gaining popularity among American consumers as a new superfood. Some sea vegetable farmers in New England have begun to distribute fresh or minimally processed sea vegetables to local restaurants and to retail distributors. However, limited knowledge about quality loss and processing effects on fresh, farm-raised sea vegetables postharvest obstructs the growth of a vibrant sea vegetable industry. The objectives of this research were to: 1) evaluate the quality changes and shelf life of four fresh sea vegetables species - dulse, *Gracilaria*, sugar kelp and winged kelp - during refrigerated storage, 2) determine the basic nutritional composition of these fresh sea vegetables, and 3) evaluate the effects of blanching and freezing on the antioxidant capacity of the aforementioned sea vegetables.

Fresh dulse and *Gracilaria* were stored at 35 ºF and 45 ºF for up to two weeks and periodically tested for sensory, microbial, physical and biochemical quality attributes.

The species exhibited opposite trends for the effect of storage temperature: the lower storage temperature resulted in a longer acceptable quality shelf life for dulse (11 days) whereas the higher temperature resulted in a longer acceptable quality shelf life for *Gracilaria* (10 days), based on sensory evaluation. For the brown sea vegetables, fresh sugar kelp (February and June harvest) and winged kelp (whole fronds and slaw) were stored 35 ºF and 45 ºF for up to two weeks and periodically tested for sensory attributes, microbial, physical and biochemical quality parameters. The lower storage temperature maintained the quality of whole fronds and shredded slaw better than the higher storage temperature. Harvest season impacted the shelf life of sugar kelp significantly, resulting in an acceptable quality shelf life of 12 days for sugar kelp harvested in June compared to a 6-day shelf life for sugar kelp harvested in February for samples stored at 35 ºF.

All four species under investigation contained ~80-90 g/100g moisture. The dry mass was rich in total minerals including potassium, calcium and magnesium but low (~2-3 g/100g) in crude lipid. The protein content was variable, with dulse containing the highest (22.1 g/100g) amount among the four species whereas winged kelp had the highest (58.4 g/100g) carbohydrate content. The highest (31.4 mg/100g) vitamin C content was found in sugar kelp whereas the lowest was found in *Gracilaria* (1.5 mg/100g).

The antioxidant capacity of blanched, frozen and blanched frozen dulse, *Gracilaria*, sugar kelp and winged kelp was compared to that of fresh samples. Blanching significantly ($p<0.05$) decreased the total phenolic content and the antioxidant capacity of the sea vegetables, however, freezing at -20 ºC for one month did not affect their TPC and antioxidant capacity in most cases. Overall, the brown sea vegetables had higher

antioxidant capacity compared to the red sea vegetables. The results of these studies provide important information for the growing sea vegetable industry in New England as well as contribute to on-going sea vegetable research.

DEDICATION

I dedicate this manuscript to the four pillars of my life, maa (Rita Nayyar), papa (Krishan Nayyar), didi (Isha Nayyar), and my fiancé, Eeshaan Asaikar.

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CHAPTER 1

OVERVIEW

Fresh, locally produced, and sustainable foods currently receive considerable attention from American consumers. With over one-third of adults in the U.S classified as obese (Ogden and others 2014), the western diet dominated by saturated fat and added sugars has been repeatedly linked to various health related disorders including obesity, diabetes, hypertension and cardiovascular diseases. In recent years, various kinds of "functional foods" including fatty fish, oats, and nuts have been in the limelight due to their ability to provide healthful nutrients to the body. Health conscious consumers want tasty foods (Holland 2016) that not only provide them with basic nutrition but also are loaded with secondary nutrients (Venugopal 2009). Consumer demands and concerns are the key drivers for minimally processed, nutrient-dense foods. The food industry has responded by looking for new products to fulfill these consumer desires. Moreover, there is a growing need for additional sources of nutrient-rich, sustainable foods (Future Food 2050) to suffice for the growing global population (United Nations 2015).

Human consumption of seaweeds, also known as sea vegetables, started centuries ago, some of the first consumers being inhabitants of coastal regions. Popular and dominant in Asian cuisine, seaweed consumption is believed to have spread to other countries as people migrated. In the US, seaweed products are available across the nation and are particularly enjoyed in Maine and Hawaii (McHugh 2003, Kilnic and others 2013). There is a growing demand for seaweed products, partly due to growing awareness of their nutritional benefits (Hotchkiss and Trius 2007) and a wider acceptance of ethnic cuisines. According to an extensive report on the seaweed industry by the Food

and Agriculture Organization (FAO), "With the current trend for consumers to embrace organically grown and natural foods from clean environments, seaweeds should receive an increasing acceptance" (McHugh 2003).

In order to meet high seaweed market demands in Asian countries such as China and Japan, seaweed aquaculture has partially replaced wild harvest to make production more sustainable (McHugh 2003). Although they represent only a small part of total aquaculture production, several species of sea vegetables are currently being tested for their aquaculture potential in Maine. As consumers seek fresh, local and farm-raised food products, fresh aquacultured sea vegetables have great potential to make their way to the market through multiple channels including food service and retail.

1.1. Introduction to Seaweed

Seaweeds are marine macroalgae. In contrast to terrestrial plants, seaweeds are not differentiated into roots, stem and leaves. Seaweeds consist of stem-like thalli, leaf-like fronds and the more evolved forms have a holdfast for anchorage (Lobban and Harrison 1997). These organisms are found in salt waters around the world attached to rocks and other hard substrata (Bold and Wynne 1985, Kilnic and others 2013). There are some species such as sea lettuce (*Ulva spp.*) and black carrageen (*Furcellaria lumbricalis*) that do not require any substrata and float freely in the ocean (Bold and Wynne 1985, Mouritsen and others 2013c). All seaweeds contain chlorophyll and are photosynthetic. Due to this, seaweeds typically grow close to the surface of water where light is abundant or at least sufficient.

Marine macroalgae are broadly classified into three divisions based on their pigmentation: Chlorophyta (Green), Rhodophyta (Red) and Phaeophyta (Brown). Several

genetic and phenotypic variations can be seen between and among the three divisions. In total, there are about 10,000 species of seaweeds; 6,200 red, 1,800 green and 1,800 brown (Mouritsen and others 2013a).

1.1.2. Rhodophyta

Red seaweeds or Rhodophyta make up the largest division among the three groups of seaweed. Examples of some red seaweeds include *Gelidiella calcicola, Palmaria palmata* (dulse), *Chondrus crispus* (Irish moss) and *Porphyra spp.* (nori). Found in the benthic region, several species of red seaweeds are used for their polysaccharides and as food. In general, red seaweeds have a higher content of protein compared to brown (Bocanegra and others 2009). Although they contain photosynthetic chlorophyll pigments, they get their dark red color due to the presence of water-soluble phycobiliprotein pigments such as phycoerythrin and phycocyanin (Gantt 1990, Lobban and Harrison 1997, Bocanegra and others 2009). Depending on the species, their color can range from a bright pink to red, to dark brown, to almost black (Cox 2012). They also contain other pigments such as carotenoids and xanthophylls (Kraan 2013).

1.1.2. Phaeophyta

Brown seaweeds or Phaeophyta, like red or green seaweeds, come in different sizes and colors. Similar to Rhodophyta, they grow in the littoral and sub-littoral region with turbulent waters. Various species of brown seaweeds, including those from genus *Laminaria (Saccharina), Sargassum* and *Fucus,* are consumed in different parts of the world. A treasure house of polysaccharides and dietary fiber, brown seaweeds are a rich source of alginates (Chapman and Chapman 1980). The dominant carbohydrate present is laminaran (El Gamal 2011). From light olive to golden brown to dark brown, their color

varies depending on the species and several environmental factors such as light intensity and pH of water (Bold and Wynne 1985, Kraan 2013). Fucoxanthin, found only in brown seaweed, masks the green color from pigments chlorophyll *a* and *b*. Some brown seaweeds grow only a few centimeters whereas some species can grow over 45m in length. Species such as kelp can form dense forests in the ocean, growing up to 60m under water (Round 1981).

1.1.3. Chlorophyta

Chlorophyta is the major division consisting of green marine macroalgae along with microalgae found in marine and fresh water environments. It evolved differently than red and brown seaweeds, the latter two restricted to marine environments. Green seaweeds can be different shades of green depending on the presence of chlorophyll *a* and *b* in the chloroplast (Bold and Wynne 1985, Bourgougnon and Stiger-Pouvreau 2012). Most of the green seaweeds are used as food in different regions of the world. Some of the key species widely consumed belong to the genus *Ulva* and are commonly known as sea lettuce.

1.2. Industrial Uses of Seaweed

Many industries have used seaweeds for various purposes including food, pharmaceuticals, nutraceuticals and cosmetics. Red and brown seaweeds, in particular, have been exploited for three hydrocolloids; carrageenan, agar and alginate (Chapman and Chapman 1980, McHugh 2003). Hydrocolloids serve as key components of numerous finished products. These water-soluble carbohydrates are primarily used as thickeners or gelling agents in toothpaste, dairy products, desserts, medicines, and lotions (Murthy and Banerjee 2012). Seaweed cultivation and the extraction processes for these

hydrocolloids have progressed tremendously since the hydrocolloid industry's inception in the early 1900s (McHugh 2003).

Red seaweeds, particularly of genus *Gelidium* and *Gracilaria*, are used to extract agar. The agar creates a gel that firms when cooled but allows the growth of bacteria. It is also used for preserving seafood, sizing of fabrics, making gum and jellies, in the alcohol industry and as a lubricant. Carrageenans, also extracted from red seaweeds, are sulfated polysaccharides used extensively in the food industry as thickening, gelling and stabilizing agents. Although initially extracted from *Chondrus crispus*, the two predominant species now used to extract carrageenans are *Kappaphycus alvarezii* and *Eucheuma denticulatum* (Chapman and Chapman 1980, McHugh 2003, Bixler and Porse 2011).

Alginates or alginic acid are extracted from brown seaweeds. Species of *Laminaria, Ascophyllum, Ekclonia* and *Durvillaea* are particularly used to extract alginates. They are used in textiles, food, paper, and the fiber industry. Their gelling property is utilized to make instant jellies and their stabilizing property is utilized to give ice cream a smooth texture. Moreover, their thickening property is used in various syrups and creams (Chapman and Chapman 1980, McHugh 2003, Bixler and Porse 2011).

Seaweeds have been used in several other industries including the fish and animal feed, biofuels, wastewater treatment, medicinal, cosmetic and pharmaceutical industries. The fish and animal feed industry has been utilizing seaweeds and their polysaccharides to serve multiple purposes in the feed. The polysaccharides bind the feed, making it easier to handle in the cage or tank whereas seaweeds as an ingredient provide ample nutritional benefits. Another area where they are receiving much-deserved attention is as

a renewable source of energy. Although less explored until recently, seaweeds are being considered as potential sources of biofuels (Wei and others 2013). Specific extracted compounds from seaweeds are known to have desired properties including anti-inflammatory, anti-bacterial and anti-fungal (El Gamal 2011). These compounds have found their place in several medicinal, cosmetic and nutraceutical applications. In addition, wastewater treatments use seaweeds to reduce heavy metals (Aderhold and others 1996) and nitrogen and phosphorous containing compounds (Davis and others 2003, McHugh 2003) from industrial waste and sewage.

1.3. Seaweed Aquaculture

The seaweed aquaculture industry was established as a result of steady growth in its demand (FAO 2012). According to FAO, in 2013, around 24,032,084 tons of seaweeds were produced through aquaculture valued at $5,470,217; compared to 14,792,817 tons valued at $3,716,724 in 2009 (FAO 2015). The demand for aquacultured seaweeds has seen an upward trend (FAO 2015). Currently, over 40 countries participate in seaweed farming. Production of red seaweeds ranks the highest at about 61.8% followed by brown at 38.1% and lastly by green seaweeds (FAO 2012).

More than 90% of seaweeds sold in the international market are grown via aquaculture in China, Korea and Japan but this has been restricted to about 20 species so far (Bocanegra and others 2009, Fleurence and others 2012). However, the world's largest producer of seaweeds, wild or aquacultured, continues to be China (FAO 2015). China's seaweed production is focused on brown alga, *Laminaria japonica*, commonly known as kombu whereas *Undaria pinnafida* makes up to over 50% of Korea's seaweed production. Nori, *Porphyra spp.,* is primarily cultivated in Japan (Bocanegra and others

2009, FAO 2015). Although some coastal communities are beginning to invest in seaweed aquaculture in the U.S., the practice is highly underexploited compared to in Asian countries.

The state of Maine has been actively involved in creating a thriving aquaculture industry to support coastal farmers since the early 1990s, in conjunction with several other institutions including the Maine Sea Grant, the University of Maine and its cooperative extension team. A big breakthrough with regard to seaweed aquaculture came in 2010, when the first seaweed crop (sugar kelp) was successfully cultivated in Maine. Subsequently, several other varieties including *Alaria esculenta*, *Porphyra umbilicalis*, and *Palmaria palmata* have been cultivated with a few others such as *Gracilaria tikvahiae*, and *Laminaria digitata* under development (Maine Sea Grant).

1.4. Seaweed as Food

Many seaweed researchers and enthusiasts believe that the term seaweed has a negative connotation associated with it (McHugh 2003) which impacts the consumer mind-set unfavorably and hinders them from trying this nutrient packed marine food. On-going debate about how to best describe seaweeds, especially those intended for human consumption, has resulted in a new, more positive term called "sea vegetables." This term is gaining acceptance particularly in the West. Although this term is gaining popularity in the seafood industry, the more common and well-accepted term among most consumers remains "seaweed". For the purpose of this thesis, the term "sea vegetable," will be used when referring to food.

Asian countries such as China, Japan and Republic of Korea are the largest consumers of sea vegetables (McHugh 2003, Kolb and others 2004). Sea vegetables have

gained in popularity across the U.S. through Asian restaurants, however, they have traditionally been consumed in Maine and Hawaii (McHugh 2003, Kilnic and others 2013). One of the most popular forms of sea vegetable in the culinary world continues to be nori sheets (*Porphyra Spp*.), used to make sushi rolls. Two other products gaining popularity in the western world are kombu (*Saccharina japonica*) and wakame (*Undaria pinnatifida*), both used in a variety of products such as stews, salad, and with other seafood. Other species such as dulse, various kelps, rockweed, pepper dulse, *Gracilaria spp.*, and sea lettuce are used in soups and salads or processed into dried snacks whereas some are pickled, toasted or eaten in jellies (Chapman and Chapman 1980, Lobban and Wynne 198, Mouritsen and others 2013a)**.** In Hawaii, a variety of sea vegetables are mixed with seafood and consumed as a condiment; as powdered spice or flakes (McDermid and Stuercke 2003). Some *Gracilaria* species, locally known as *limu*, are consumed as a garnish as well (Paull and Chen 2008). However, sea vegetable intake in North America is limited, compared to Japan, where approximately 5.3 g/person of sea vegetable are consumed daily (Matsumura 2001).

The food industry follows consumer demands in the development of new products and recognizes that functional foods continue to gain popularity among the masses, especially among millennials and baby boomers (Venugopal 2009, Prepared Foods 2015). Due to their high mineral, vitamin, antioxidant and fiber content, sea vegetables are often considered as functional foods, especially in the West (Bocanegra and others 2009, Venugopal 2009, Ferraces-Casais and others 2012, Mohamed and others 2012).

In the quest to find additional food resources that provide ample nutrition yet are environmentally and ecologically sustainable, a variety of seafoods including sea

vegetables have been examined (Tarver 2015). The ongoing development of sea vegetable aquaculture, health benefits associated with sea vegetables and the demand for new functional foods provide a great platform for the development of new sea vegetable products. In the past decade, many experts have predicted sea vegetables to be the next superfood (Holland 2016) with a popularity rivaling kale or avocados.

Out of 221 species of sea vegetable harvested all over the world, 66% are used by the food industry (Zemke and Ohno 1999). Out of the sea vegetables harvested, most are used for human consumption, eaten in various forms, particularly in Asian countries (Zemke and Ohno 1999, Bocanegra and others 2009, Fleurence and others 2012). A spike in 2011 and steady increase since then, in the search term "seaweed snacks" indicates a rising interest in alternative healthy snacks among consumers (Spiegel 2014). However, despite its popularity its production, processing and consumption is limited to a few countries, species and forms (Bocanegra and others 2009, Redmond 2012).

Although China, Japan and Korea are the top producers of sea vegetables, other countries including Indonesia, the Philippines, Malaysia, Vietnam and India also produce and consume them in smaller amounts (FAO 2012). Various species of sea vegetables are incorporated in rice, noodles, broths and soups to impart *umami* flavor. Moreover, sea vegetable flavor is used as a common ingredient in other snacks such as potato or corn chips. Use of sea vegetable is widespread in East Asian cuisine.

Although many countries in Europe are coastal, the practice of incorporating sea vegetables in the human diet has been minimal (Fleurence and others 2012). France is the largest sea vegetables producer and the only country in Europe with established regulations on sea vegetable consumption (Fleurence 1999, Bocanegra and others 2009).

Along with dried snacks and seasonings, a company in France called Les Ouessantines also sells canned and salted sea vegetables (Les Ouessantines). In other European countries such as Spain, Ireland, Germany and Denmark, sea vegetables consumption has not reached far from coastal communities and is limited to only a handful of companies (Cox 2012).

In the U.S., efforts are being made by a number of small businesses to create and sell diverse sea vegetable products. Around 250 species of sea vegetables are found across the Gulf of Maine. Although all are edible, only 11 species are currently being harvested for food (Maine Sea Grant). Various dried forms including whole leaf, flakes and coarse granules of nori, kombu, winged kelp, bladder wrack, dulse, irish moss and sea lettuce are being sold online and in some health stores (Redmond 2012, Maine Coast Sea Vegetables, Vitamin Sea Seaweeds). Another sea vegetable company called Sea Snax is selling sea vegetable chips, "stix," flakes and "seaweed sprinkles" in interesting flavors including sesame, almond, barbeque and wasabi (Sea Snax). Dried sea vegetables are also used as seasonings in combination with other flavors (Maine Sea Coast Vegetables). Some companies are selling a dried sea vegetable with salad mix, to be eaten after it is reconstituted with water (Maine Seaweed). On the Pacific coast, *Gracilaria spp.*, also known as limu, along with few other species are used in some traditional preparations (Chapman and Chapman 1980, McDermid and Stuercke 2003, Paull and Chen 2008).

Several companies are marketing fresh or minimally processed sea vegetable dishes such as Asian salads or ready to eat combinations. To attain better shelf life, mildly salted versions of fresh sea vegetable are also being sold. In efforts to make highly

perishable fresh products stay longer, some companies have come up with fresh frozen forms of salads. The shelf life, processing techniques and distribution methods of fresh sea vegetables are poorly investigated and hence, pose challenges to develop new products (Redmond 2012).

1.5. Health Benefits of Sea Vegetables

A high calorie diet in addition to lower physical activity has been linked to increasing incidences of several disorders including obesity, cardiovascular disease (CVD) and type 2 diabetes in developed countries (Selassie and Sinha 2011). One-third of the adults and 17% of the youth are obese in the U.S. (Ogden and others 2014). On the contrary, low incidences of coronary heart diseases (CHD) & obesity and higher life expectancy in the Japanese is often attributed to their high consumption of seafood products including sea vegetables (Iso 2011, Brown and others 2014).

Brown and others (2014) reviewed numerous in vivo animal, in vitro and epidemiological studies in a recent paper concluding that although more robust studies are required, bioactive compounds including pigments and dietary fiber along with lipids high in omega-3 fatty acids from sea vegetables may have potential advantages for human health. These may include better weight management and lower chances of cancer, CHD, CVD and diabetes. Anti-microbial and anti-viral properties of sea vegetables are also being tested in vivo and in vitro to combat diseases such as HIV and herpes.

Sea vegetables can be consumed to target specific diseases or to maintain positive health status. They are low in calories and high in vitamins, minerals, dietary fiber, antioxidants and other bioactive compounds making sea vegetables attractive to

researchers and consumers (Bhuvaneswari and others 2003, Brown and others 2014, Kilnic and others 2013).

1.6. Nutritional Composition of Sea Vegetables

Nutritional composition of sea vegetables is dependent on various intrinsic and extrinsic factors. The quantity of chemical constituents in sea vegetables is governed by multiple factors such as growing season (Hernandez-Carmona and others 2009, Schiener and others 2015), pH, light, temperature and salinity (Baghel and others 2014). Fresh sea vegetables typically contain about 70 to 95% of water (Wong and Cheung 2001, McDermid and Stuercke 2003). However, as previously mentioned, they are typically dried before consumption. Hence, most of the literature has reported nutrient content on a dry weight basis (dwb). Further sections discuss major constituents and selected micro nutrients of sea vegetables.

1.6.1. Total Minerals

Seaweeds are considered high in minerals and trace elements. This has made them a good contender for inclusion in supplements and nutraceuticals (Mišurcová and others 2011). Marine macroalgae absorb minerals and other nutrients from their surroundings. The presence of a cell wall filled with a polysaccharide matrix enables them to store these macro and micro-elements (Davis and others 2003). The chemical composition of these walls has a huge effect on absorption of these elements, resulting in varying amounts of minerals within sea vegetables of the same genus (Davis and others 2003, Mišurcová and others 2011). Sea vegetables have a greater ability to absorb rare earth elements in comparison to their terrestrial counterparts (Mišurcová and others 2011).

Table 1.1. Proximate composition of selected sea vegetables (%, dwb).

Species	Ash	Crude Protein	Crude Fat	Carbohydrate
Brown Sea vegetables				
Saccharina spp. (Laminaria spp.)	19.2-28.8	5.0-6.7	0.8-1.6	49.1
Fucus spp.	20.9	3.0-11.1	2.7	70.3
Alaria esculenta	24.5	9.1	1.5	64.9
Undaria pinnatifida	21.2-32.8	12.7-14.1	1.5-2.7	47.8
Red Sea vegetables				
Palmaria palmata	42.2	12.3	1.4	44.1
Gracilaria spp.	17.8-53.4	7.9-10.5	0.1-2.1	58.4
Porphyra spp.	8.5-8.7	33.0-47.0	0.7-1.6	40.5

Adapted from McDermid and Stuercke (2003), Bocanegra and others (2009) and Maehre and others (2014).

Ash content in sea vegetables can range from 8 to 55% of algal dry weight (Table 1.1) (Ito and Hori 1989, Rupérez 2002, McDermid and Stuercke 2003, Baghel and others 2014). Multiple studies have reported higher ash content in red sea vegetables compared to brown sea vegetables, ranging from 22.7 to 53.4 % (dwb) and 28.9 to 32.0 % (dwb), respectively (McDermid and Stuercke 2003, Baghel and others 2014). On the contrary, a study conducted on red and brown sea vegetables in Spain found the ash content in brown sea vegetables to be higher than in red sea vegetables (Rupérez 2002). Depending on the species analyzed, these values can differ considerably. A review paper on red sea vegetables reported ash values to range from 11.7 to 36.6 % (dwb) (Morgan and others 1980).

A recent study by Astorga-Espana and others (2015) concurred with previous literature, concluding that genera, species, season and geographic location affect the amount of total minerals present as well as the concentration of specific minerals in sea

vegetables. Seventy-three sea vegetable samples from different genera, family and genus were tested from three different regions in the sub-Antarctic eco-region. Various other factors such as physiological stress, pH, salinity of water and other environmental changes have also been reported to influence mineral deposition in sea vegetable (Rao and others 2007, Kumar and others 2008, Mišurcová and others 2011, Baghel and others 2014, Astorga-España and others 2015).

1.6.1.1. Specific Minerals

Macro-elements found in noticeable concentrations in sea vegetables are sodium (Na), potassium (K), phosphorus, calcium and magnesium (Table 1.2) (MacArtain and others 2007, Rao and others 2007, Astorga-España and others 2015). High concentrations of sodium and potassium are found in several species but the Na/K ratio is usually below 1.5 (MacArtain and others 2007, Rao and others 2007), which is much lower compared to vegetable broth (15) and olives (81) (USDA 2014). The Na/K ratio in some brown algae such as *Padina pavonica*, *Dictyota dichotoma*, and *Colpomenia sinuosa* was found to be below 0.5 (Tabarsa and others 2012a). Essential microelements such as iron, manganese, copper and zinc, important for maintaining homeostasis in the human body, are also present in sea vegetables (Rao and others 2007, Mišurcová and others 2011, Astorga-España and others 2015).

Seaweeds have also attracted attention for being high in iodine. In general, brown sea vegetables are relatively richer in iodine than red and green sea vegetables. Countries combating mineral deficiencies should look deeper into incorporating sea vegetable in their diet. However, extremely high intake of sea vegetables in Japan results in iodine consumption of approximately 1-3 mg/day, which may lead to thyroid disorders (Teas

and others 2004, Zava and Zava 2011). In the U.S., the dietary reference intake for iodine is 0.15 mg/day (Teas and others 2004, Mišurcová and others 2011).

Table 1.2. Mineral content of selected sea vegetables (mg/100g, dwb)

Mineral	*Saccharina spp.*	*A. esculenta*	*P. palmata*	*Gracilaria spp.*	*Porphyra spp.*
Calcium	800-1000	800	360	255-948	430-830
Potassium	2840	N/A	N/A	11170-11380	450
Magnesium	10-840	870	270	438.5	12-960
Phosphorus	120-210	230	270	N/A	73-350
Iron	5.8-12	8.7	10	29-67	13-20
Sodium	1830-3818	N/A	N/A	4105-10356	790
Zinc	2.2-6.0	4.9	2.9	6.33	31
Copper	0.1	0.2	0.5	0.6-0.9	2.0-2.8

Adapted from McDermid and others (2003), Bocanegra and others (2009), Tabarsa and others (2012), Baghel and others (2014) and Maehre and others (2014). N/A= missing data.

1.6.2. Carbohydrates

In the recent past, a lot of attention has been given to carbohydrates found in sea vegetables. This interest is attributed to their various functions as food ingredients including thickening, stabilizing emulsions and formation of gels. Carbohydrates may form the highest percentage of the dry matter in sea vegetables, reaching up to 70% in some kelp species (Ortiz and others 2006). However, *Sargassum polycystum*, another brown sea vegetable, contained 33% carbohydrates (dwb), even higher than the red sea vegetable, *Eucheuma cottonii*, which had 26% carbohydrate (dwb) (Matanjun and others

2009). Schiener and others (2014) also concurred with previous literature, finding approximately 70% carbohydrates (dwb) in four species of brown sea vegetables.

From a nutritional point of view, dietary fibers within the carbohydrate fraction have been of keen interest to researchers. Dietary fibers cannot be digested by humans but add to the bulking effect and aid to maintain the gut microflora. Hence, in the last 10 years, numerous studies have focused on determining total dietary fiber in sea vegetables, as well as soluble and insoluble fractions. In some cases, total dietary fiber can account more than 50% of the dry mass of the sea vegetables (Wong and Cheung 2000).

1.6.3. Proteins

Proteins are an essential part of the human diet, contributing to energy and structure in addition to performing biochemical and cellular functions in the body. Protein deficiency can have serious implications including malnutrition and retarded growth. Although in terms of protein quality and digestibility animal products rank higher, plant proteins have received much attention despite lacking one or more essential amino acid(s) (EAA) in their profile (Cerna 2011). Similarly, proteins from a plethora of different sea vegetables have been evaluated for their content, value, bioavailability and digestibility.

Protein content in sea vegetables not only varies due to environmental factors but also due to the method of extraction and detection (Fleurence 1999, Mišurcová and others 2011, Angell and others 2015). Indirect methods of protein content analysis detect all nitrogenous compounds in the sample, including nitrogen present in free amino acids, DNA and chlorophyll, often overestimating protein in sea vegetables (Lourenço and others 2002, Angell and others 2015). A thorough study by Angell and others (2015)

suggested a revised nitrogen-to-protein conversion factor of 5 for sea vegetables, instead of the universally accepted 6.25. However, most of studies discussed here used 6.25 to determine protein content of sea vegetables.

While assessing nutritional value of eight different sea vegetables, Patarra and others (2011) reported higher protein content for sea vegetables belonging to Rhodophyta and Chlorophyta than Phaeophyta. These results concur with previous studies in which red and green sea vegetables contained a higher percent of crude protein (10-47% dwb) in comparison to brown sea vegetables (3-15% dwb) (Mabeau and Fleurence 1993, Galland-Irmouli and others 1999, Burtin 2003, Misurcova 2011). Nori had the highest amount of crude protein compared to 21 other species from Hawaii (Table 1.1) (McDermid and Stuercke 2003). However, wakame, a very popular brown sea vegetable, has repeatedly been reported to have higher than 15% of crude protein (Mabeau and Fleurence 1993, Dawczynski and others 2007).

Protein content of dulse can reach 35% (dwb) in some cases, but Galland-Irmouli and others (1999) reported the yearly average as 18.3%, with the highest readings in the winter and lowest in the summer. This seasonal variation in dulse and other red sea vegetables has been previously attributed to varying nitrogen content of sea water and the intensity of light (Morgan and others 1980), resulting in destruction or loss of water-soluble phycobiliproteins present in red sea vegetables.

Humans are unable to synthesize certain amino acids, called essential amino acids (EAAs) making diet the primary source of such amino acids. Nine out of twenty common amino acids fall under this category. Sea vegetables may contain all amino acids (Matanjun and others 2009), but their presence and content differ depending on the

species and other factors mentioned earlier. In certain cases, EAAs may comprise up to 49.7% of the total amino acids present (Lourenço and others 2002), higher than that of soybean as reported by Galland-Irmouli and others (1999). Moreover, Mabeau and Fluerence (1993) found that the amino acid composition of *P. tenera* was comparable to that of ovalbumin (or egg white), often used as a standard to compare protein quality. In comparison to terrestrial plants, macroalgae have a higher protein quality (Maehre and others 2014). These findings suggest that sea vegetables can add value to the human diet, in particular for vegans.

1.6.4. Crude Fat

Macroalgae are known for their low lipid content, making them appealing to certain health-conscious consumers. In general, the lipid fraction can be anywhere between 1- 3% of dry mass (Table 1.1) (Mabeau and Fleurence 1993, Bocanegra and 2009). One of the studies evaluating nutritional composition of nori (*P. purpurea*) and wakame (*U. pinnatifida*) found 1% and 2.7% (dwb) lipid content, respectively, falling within the previously reported range for sea vegetables (Taboada and others 2013). McDermid and Stuercke (2003) reported crude lipid content of 14 Rhodophyta from Hawaii to be less than 5% (dwb) whereas two out of four brown sea vegetables contained over 15% (dwb) of crude lipid. This indicates that different species from the same geographic location can differ in nutritional composition.

Total crude fat analysis in thirty-four brown and red sea vegetables (17 each) from Asia showed no significant differences ($P>0.05$) between the two algal classes. However, significant differences ($P<0.05$) were found in the lipid content among selected species. Wakame (*U. pinnatifida*) and *Porphyra spp*. from Japan and Korea had higher

lipid content than *Porphyra spp*. from China, kelp (*Laminaria spp.*) and Hizikia fusiforme, indicating species and geographic location can affect overall lipid content (Dawczynski and others 2007).

1.6.4.1. Polyunsaturated Fatty Acids (PUFAs)

Fatty acids are generally divided into saturated or unsaturated, and are typically comprised of an even number of carbon atoms ranging from 14-24 carbons (McClements and Decker 2008). Polyunsaturated fatty acids (PUFAs) are unsaturated fatty acids that contain more than two double bond between carbon atoms (McClements and Decker 2008). They have received a lot of attention due to their positive effects on human health. Amongst them, intake of essential omega-3 and omega-6 fatty acids, in particular, have been linked to constructive help in disorders including obesity, CVD, mental and behavioral health (Cichon 2003, Ruxton and others 2007). It is important to consume both types of essential FAs in equal amounts. However, the western diet, being rich in dairy and vegetable oil, provides more ω-6 than ω-3 fatty acids (Simopoulos 2008).

Interestingly, most of the fatty acids content in macroalgae is comprised of PUFAs, sometimes over 50% of total fat content (Matanjun and others 2009, Mohamed and others 2012). To our benefit, the ω-6/ω-3 ratio found in sea vegetables is often around 1 (MacArtain and others 2007, Tabarsa and others 2012a, Boulom and others 2014), much lower compared to vegetable oils such as safflower (77:1) and corn (60:1) (Simopoulos 2001). Extremely low ω-6/ω-3 ratios were also seen in *U. pinnatifida* (0.37) and *P. purpurea* (0.10) from Spain (Taboada and others 2013). Long chain ω-3 fatty acids, found in sea vegetables, such as docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) have been associated with several health benefits leading to

pharmaceutical and nutraceutical applications (Ruxton and others 2007, Kumari and others 2010). Dawczynski and others (2007) found that out of numerous species analyzed, *Hizikia fusiforme* and a few red algal species were very rich in ω-3 fatty acids, amounting to over 50% of fatty acid methyl esters.

Differences in seasons of the year bring about changes in PUFAs quantity as well (Nelson and others 2002, Robertson and others 2013, Boulom and others 2014). Wakame (*U. pinnatifida*) showed seasonal differences in PUFA content and ω-6/ω-3 ratio from July to December, being the highest in winter (Boulom and others 2014). Temperature has proven to influence fatty acid profile greatly in sea vegetables, among other factors (Robertson and others 2013).

1.6.5. Vitamin C

Sea vegetables are also well recognized for their vitamin content. They contain water soluble vitamins B and C, (Mabeau and Fleurence 1993, MacArtain and others 2007, Miyamoto and others 2009) and fat soluble vitamins A (precursor beta-carotene), K and E (MacArtain and others 2007, Mouritsen and others 2013b). Other than providing essential nutrients to humans, these vitamins play biological roles in sea vegetables as well. Some of these vitamins such as vitamin C and E also function as antioxidants and may be present due to exposure to physiological and/or environmental stress (MacArtain and others 2007). Although sea vegetables are photosynthetic and are assumed to be autotropic, several species require certain B vitamins from the environment to grow. Vitamin B_{12} (cobalamin) and vitamin B_1 (thiamine) were found to be essential nutrients in 56 and 19 of 161 species tested, respectively (Croft and others 2006).

A study of nine sea vegetables from Vietnam found the highest vitamin C content in the green sea vegetable, *Ulva reticulata.* In comparison to vitamin C content of raw carrots, 59 µg /g, it contains approximately 2.5 times more, 145.6 µg/g (Hong and others 2007). Furthermore, all the nine species, *U. reticulata, Caulerpa racemose, Gelidiella acerosa, Laurencia obtuse, Gracilaria tenuistipitata, Hypnea valentiae, Porphyra crispate, Kappaphycus alvarezii,* and *Sargassum mcclurei,* contained more vitamin A, 2.12 µg/g, 2.16 µg/g, 0.85 µg/g, 0.58 µg/g, 2.10 µg/g, 0.57 µg/g, 0.90 µg/g, 1.06 µg/g, and 0.96 µg/g, respectively, compared to 2% reduced milk fortified with vitamin A, 0.56 µg/g (Hong and others 2007, USDA 2014). In their study on bioactive compounds, Ferraces-Casais and others (2012) found that *Himanthalia* contained the highest amount of vitamin C followed by nori, kombu and dulse. It is also noteworthy that water soluble vitamins are extremely sensitive to light and heat. Different processing conditions often lead to partial or complete loss of such vitamins. Hence, if sea vegetables are consumed with the intention of supplementing vitamins in the diet, fresh or minimally processed products may be of greater use.

1.6.6. Bioactive Compounds

Bioactive compounds are essential and non-essential types of compounds including polyphenols, antioxidants, and vitamins that exist in foods and provide health benefits beyond the basic nutritional value of the product (Biesalski and others 2009). A strong correlation between consuming foods high in bioactive compounds and good health has been demonstrated in numerous recent publications (Zubia and others 2009, Cornish and Garbary 2010). Some specific examples of bioactive compounds found in food are tocopherols, vitamin C, glutathione, carotenoids and flavonoids. The food,

nutraceutical, pharmaceutical and cosmetic industries are beginning to give ample attention to secondary metabolites in sea vegetables due to the potential benefits associated with them. The presence of diverse compounds with high biological activity in different species of sea vegetables is often attributed to their unique marine environment, which causes high physiological stress.

Lipid containing foodstuffs are subject to oxidation. Lipid oxidation often results in off flavors, rancidity and overall deteriorated quality of food material. Exposure to light, metal ions and heat can lead to autoxidation of PUFAs creating reactive oxygen species (ROS) (Karlsdottir and others 2014). Antioxidants are substances produced by organisms to defend their cells against free radical-induced oxidative stress. These substances scavenge for reactive oxygen species (ROS) thereby reducing oxidation (Valko and others 2007). There are several studies that suggest oxidative stress is one of the major factors in aging and can lead to age-related disorders. Hence, a strong correlation between consuming foods high in antioxidants and good health has been tested time and again in the scientific literature (Zubia and others 2009, Cornish and Garbary 2010). Although the role of antioxidants in slowing down aging has been questioned by some researchers (Sohal and Orr 2012), antioxidants continue to attract consumer attention.

In the past few years, natural plant antioxidants have been heavily researched as replacements for commonly used synthetic ones such as butylated hydroxy toluene (BHT) and butylated hydroxy anisole (BHA) (Bhuvaneswari and others 2013). Various species of sea vegetables have been studied for their biological activity and potential applications in the food industry as food additives. Numerous compounds present in sea

vegetables contribute to their antioxidant activity including pigments (chlorophylls, carotenoids, phycobilins, xanthophylls), phenolic compounds (flavonoids, tannins, tocopherols), vitamins and their precursors along with sulfated polysaccharides (Duan and others 2006, Kuda and others 2007, Cornish and Garbary 2010, Cox 2012, Ferraces-Casais and others 2012).

Fucoxanthin, the major pigment in brown sea vegetables, has been demonstrated to induce apoptosis in human prostate cancer cells and to attenuate weight gain in white adipose tissue, in vitro (Ferraces-Casais and others 2012). Antioxidants found in brown sea vegetables include sulfated polysaccharides such as fucoidan, sulfated glycosaminoglycan, vitamins C and E, phenolic compounds such as terpenoids, and polyphenols such as phlorotannins (Cornish and Garbary 2010). Various species of red macroalgae provide antioxidants such as carotenoids (antheraxanthin, lutein, violaxanthin, xanthophylls, zeaxanthin), phycobilin pigments (phycoerythrin and phycocyanin), polyphenols such as flavonoids, sulfated polysaccharides and vitamin A (Cornish and Garbary 2010, Ferraces-Casais and others 2012).

Dulse extracts were successful in scavenging $OH^{\bullet}$ radicals in a deoxyribose assay and ROS with free radicals by the inhibition of lipid peroxidation, showing their potential as antioxidants (Yuan and others 2005a). This intertidal species protects itself endogenously from UV-induced lipid oxidation which is one of the major contributors to its antioxidant potential. Its antioxidant potential is further demonstrated through its reducing activity and total polyphenol content. The study also mentioned that the extract quenched $DPPH^{\bullet}$ and $ABTS^{\bullet}$+ free radicals in vitro and that it exhibited antioxidant

activity for a long period of time. This finding can be beneficial in many food industry applications to extend product shelf life (Yuan and others 2005a).

A study performed on tropical marine macroalgae evaluated 23 red sea vegetable species and found that all tested species showed at least some level of antioxidant activity (Zubia and others 2007). Out of the 23 species evaluated, the authors found that *Chondria baileyana* had the highest antioxidant activity with the lowest effective concentration 50 (EC_{50}) and the highest phenolic content as well. The inhibition of lipid peroxidation by *C. baileyana* extract was found to be equivalent to BHT. Various *Gracilaria* species, commonly consumed in Hawaii and the Caribbean, showed very low antioxidant activity in comparison to other red sea vegetables (Zubia and others 2007).

On the contrary, Bhuneswari and others (2013) found that phenolic content and antioxidant activity of two species of marine red algae, *Chondrococcus hornemanni* and *Spyridia fusiformis* on the DPPH radical was low in comparison to BHT and ascorbic acid. However, the authors further mentioned that increased concentration of samples and standards affected the scavenging of the DPPH radical significantly. Thus, indicating these two species of macroalgae are good sources of natural antioxidant (Bhuvaneswari and others 2013).

Various red macroalgae species from the Atlantic and Mediterranean coasts of Morocco were evaluated for their antioxidant activity using aqueous and methanol extracts of the samples (Bouhlal and others 2013). The study concluded that out of the ten species tested, the highest antioxidant activities were observed in aqueous extracts of *Asparagopsis armata* and *Boergesenia thuyoides* with 68% and 35% inhibition of the hydroxy radicals (OH), respectively. In addition to this, *Pterosiphonia complanata* had

the highest antioxidant activity against the peroxide and DPPH radicals when extracted with methanol (Bouhlal and others 2013).

Zubia and others (2007) found that *Lobophora variegata* had the highest DPPH radical scavenging activity and greatest reducing activity, which was significantly higher than that of alpha-tocopherol. Another study conducted by Zubia and others (2009) reported that *Halidrys siliquosa* extracts showed antioxidant activity significantly equivalent to that of BHT and BHA. A positive correlation between antioxidant activity and phenolic contents was demonstrated upon further fractionation of the crude extracts of high antioxidant activity species (Zubia and others 2009).

A study of four different species of brown sea vegetable was performed by de Quirós and others (2010) to measure total polyphenol content and identify selected pigments. *H. elongata* exhibited the highest polyphenol content followed by *U. pinnatifida*, *Laminaria spp* and *Laminaria saccharina,* in that order. Pigments identified using a spectrophotometric method were fucoxanthin, beta-carotene, chlorophyll *a* and phaeophytin *a*. The study concluded that the presence of these pigments and water-soluble antioxidants in these edible macroalgae make them an excellent source of antioxidants (de Quirós and others 2010).

In general, brown sea vegetables are reported to have higher antioxidant activity than red sea vegetables. However, the reducing activity of dulse extract was greater than any of the brown kelps studied by Yuan and Walsh (2006). The reducing activity followed this trend, *Palmaria palmata* (dulse)>*Laminaria setchellii*>*Macrocystis integrifolia*>*Nereocystis leutkeana*. According to this paper, the total polyphenol content of the dulse extract was 3.24-fold greater than that of *M. integrifolia* and *N. leutkeana.*

Amongst the four species, the lowest total polyphenol content was in *L. setchellii* extract. The paper concluded that the greater reducing activity of the dulse extract was associated with the generally greater L-ascorbate content in red sea vegetables in comparison to brown sea vegetables. Furthermore, the lower total polyphenol contents of the three kelp extracts were linked to oxidation and polymerization of phlorotannins in these sun-dried macroalgae (Yuan and Walsh 2006).

Ulva sp. (green sea vegetable)*, Sargassum sp* (brown sea vegetable). and *Porphyra spp.* (red sea vegetable) were compared for their antioxidant activity and total polyphenol content (Garcia-Casal and others 2009). *Sargassum sp*. showed higher total polyphenol content compared to the other two species The study also noted that the TPC (in gallic acid equivalents) was up to seven and three times greater for *Sargassum sp*. than for *Ulva sp*. or *Porphyra sp.*, respectively. *Sargassum sp*. also scored highest in antioxidant capacity, which was around double that of the other two species tested. In this study, the brown sea vegetable scored much higher in both antioxidant activity and total polyphenol content (TPC), compared to the green and red sea vegetable (Garcia-Casal and others 2009).

Jimenez-Escrig and others (2001) conducted a study on three species of fresh and two species of processed edible sea vegetable. Antioxidant activity of these species was measured using various assays such as the DPPH free radical-scavenging assay, the ferric reducing antioxidant power (FRAP) assay and the in vitro copper-induced oxidation of human low-density lipoprotein assay. The authors concluded that brown sea vegetable had much better scavenging activity compared to red sea vegetable. In addition, they also mentioned that fresh sea vegetable had higher antioxidant capacity than commercially

available dried sea vegetable, suggesting that processing and storage may have affected antioxidant capacity.

Thermal processing such as open air or oven drying may affect the macro and micronutrients present in sea vegetables (Chan and others 1997, Wong and Cheung 2001). Reduced total phenolic content and antioxidant capacity were reported by Gupta and others (2011) in *Himanthalia elongata*, which was subjected to varying drying temperatures for 24 h, and compared to fresh products. However, the authors also reported that phenolic content and antioxidant capacity increased after drying for only 2 h compared to fresh, explaining that it may have been due to increased phenolic compounds produced in response to wounds caused by increased temperature. Another study on brown algae, *U. pinnatifida* (wakame), found over a 50% reduction in fucoxanthin content and scavenging activity of the blanched then oven-dried samples versus the fresh samples (Fung and others 2013).

Sea vegetable producers are interested in developing recipes including 'ready to eat' blanched and salted fronds, frozen prepared soups and fresh or frozen salads. However, there is little information on how common processing treatments such as blanching and freezing might affect the bioactive compounds. Some producers are also looking to make use of the stipe (stem-like) portion of certain sea vegetables such as sugar kelp and winged kelp. Previous studies have shown that levels of antioxidant activity may vary in different plant tissues such as blade, stipe and holdfasts (Connan and others 2006).

Copious information on bioactivity of wild and dried forms of sea vegetables is available. However, geographical location, species and season, among other factors,

affect their concentrations, and studies on aquacultured species are needed to asses if any differences exist compared to wild harvested products. Processing effects on bioactivity of sea vegetables have scarcely been studied and deserve more attention to support sea vegetable producers interested in pursuing food processing options other than drying.

1.7. Species in focus

This section elaborates on the four species (two brown and two red) of sea vegetables investigated in this thesis project, all of which are currently being farm-raised along the coast of Maine.

1.7.1. Winged kelp (*Alaria esculenta)*

Popularly known as Atlantic wakame or winged kelp, *Alaria esculenta* is a brown macro-alga found in Atlantic waters. In countries including Ireland, Scotland, France, Canada and U.S (Maine), winged kelp is enjoyed fresh or cooked in salads or snacks (Pomin 2011, Mouritsen and others 2013c). This kelp looks different from others because it consists of a holdfast, a thick stipe which has sporophylls attached to it and fronds with a thick midrib (Fig 1.1). The olive colored blades, spreading like wings from the midrib, are usually thin and translucent (Mouritsen and others 2013c). Fresh or rehydrated sheets are used for salads or soups. Dried powder for smoothies and flakes or seasonings have also been developed and sold by a few companies, including in Maine.

Figure 1.1. Winged kelp

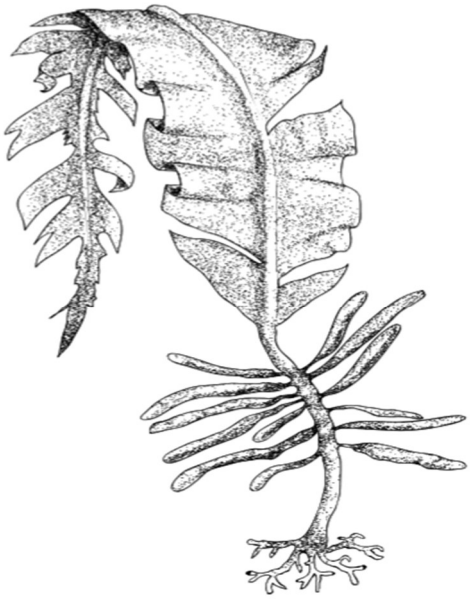


Image from Mouritsen and others (2013a)

In fresh *Alaria*, moisture content can reach up to approximately 85% (Schiener and others 2015). Maehre and others (2014) and Schiener and others (2014) reported the ash content to be close to 25% and protein content to be 9.1% and 11% of the dry mass of *A. esculenta* from Norway and Maine, respectively. Lipid makes up a very small fraction (~1.5% dwb) of the nutrient composition (Maehre and others 2015). Although there have been various studies on different species in the kelp family, literature on chemical constituents of *A. esculenta* has been scarce.

1.7.2. Sugar kelp *(Saccharina latissima)*

Saccharina latissima, a brown alga formerly known as *Laminaria saccharina*, belongs to the kelp family and is commonly referred to as sugar kelp due to its sweet flavor. Its color ranges from olive brown to deep brown. Fig 1.2 shows the structure of kelp species which have a holdfast to provide anchorage and gas bladders to aid in

floatation. There has been recent interest in studying best cultivation and grow out methods for sugar kelp in order to meet the current demand. However, only limited work has been done to assess its nutritional composition.

Figure1.2. *Laminaria spp.*

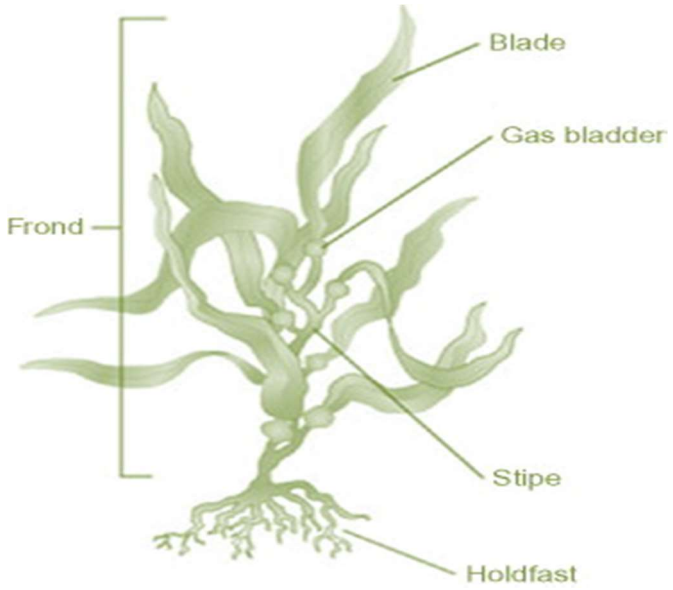


Image from Kim and Bhatnagar (2011)

Environmental factors including light intensity, temperature and availability of nutrients such as nitrates and phosphates determine the reproduction and growth rates of sugar kelp, with the favorable seasons being winter and spring (Parke 1948). Seasonal variation of sugar kelp in ash, crude protein, mannitol, laminarin and alginic acid contents was first reported by Black (1950). The author also reported that laminarin, a key component of the polysaccharide fraction of this species, is missing in the stipe portion and present in the fronds for only part of the year.

A study on seasonal variation of protein content and amino acid profile of *S. latissima* reported results similar to Black (1950), where the highest protein content was observed in November whereas the lowest was found in May-July. It is important to

note that the usual harvesting season for *S. latissima* begins in late winter to early summer, with the winter crop usually covered with epiphytes as summer progresses, rendering it unfit for human consumption. Epiphytes are small plant growths on sea vegetables that do not cause any harm to the host but may affect its acceptability as human food tremendously. Alternatively, the late autumn harvest may be used for fish or animal feed (Marinho and others 2015).

Schiener and others (2015) also looked at the seasonal variation in chemical constituents of sugar kelp harvested in Maine. On average, moisture and ash content of sugar kelp amounted to 85% (wwb) and 31% (dwb), respectively. This species was high in metal ions, with potassium, calcium, magnesium and sodium found. Alginate, which made up the major fraction of carbohydrates, was 28% (dwb) whereas laminarin was found to be 8% (dwb). Protein content varied seasonally as well with an average of 7.1% across the year. The authors also reported the polyphenol content to be 0.41% (dwb) with the highest content between May and July (Schiener and others 2015).

1.7.3. Dulse (*Palmaria palmata)*

Dulse, found in cold Atlantic waters, is one of the few red sea vegetables enjoyed in the West for centuries. When fresh, dulse color ranges from purple to dark brownish-red, while drying causes the loss of some water-soluble pigments, turning the sea vegetable to a lighter shade. Unlike in larger kelps, the holdfast used to attach dulse to rocks or larger kelp species is delicate and the stipe hardly noticeable (Fig 1.3). The fronds have a leathery texture and may grow up to 50 cm long, often making an irregular palm-like structure. The wild and aquacultured species may differ in appearance; the

latter often having fronds growing equally in all directions (Morrissey and others 2001, Braune and Guiry 2011, Mouritsen and others 2013a).

Figure1.3. Dulse frond

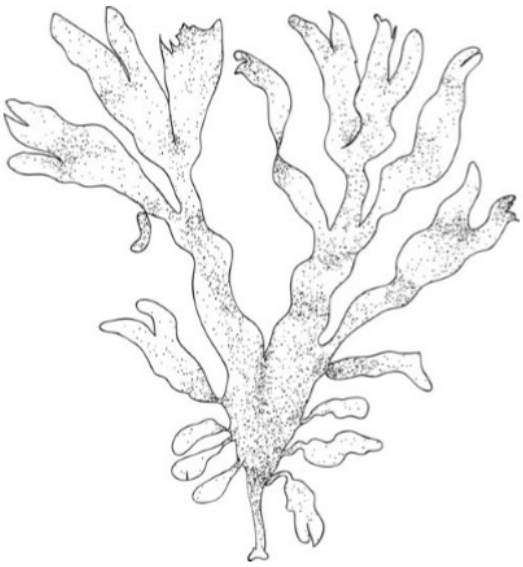


Image from Mouritsen and others (2013b)

Fresh dulse can be used in salads but drying or toasting brings out its nutty flavor, and increases its palatability. Dried, crispy dulse is enjoyed as a snack with beer or mixed with butter to go with bread. In some cuisines, people have added dried dulse granules or powder to flour. Parched dulse products are made by reabsorbing partial moisture in dried dulse, which leaves them softer with approximately a year of shelf life (Mouritsen and others 2013a, 2013b). Some research chefs have experimented with dulse as a whole ingredient, incorporating it in food products such as fresh cheeses, ice-cream and bread dough (Mouritsen and others 2012).

Moisture can account for up to 83% of fresh dulse weight. It has a relatively very high protein content (8-35% dwb) compared to other sea vegetables (Morgan and others 1980, Fleurence and others 2012, Mouritsen and others 2013b). Seasonal changes and

nitrogen content in the water play a significant role in the fluctuating protein and amino acid contents. A high nitrogen content is observed in winter through early spring, plummeting in summer and autumn. Seasonal changes also dictate concentrations and presence of specific amino acids. For example, Galland-Irmouli and others (1999) reported lysine and threonine were missing from *P. palmata* in the summer. Overall, *P. palmata* contains all amino acids except for cysteine (Morgan and others 1980, Fleurence 1999, Mouritsen and others 2013a). Additionally, short heat treatment has shown to increase the bio-accessibility of amino acids in this sea algae (Maehre and others 2015).

Lipid content ranges from 0.3 to 3.8% depending on spatial, seasonal and other factors (Morgan and others 1980, Mouritsen and others 2013b). Unlike other alga, *P. palmata* contains higher amounts of demosterol instead of cholesterol (Morgan and others 1980). Like other sea vegetables, the lipid content of dulse contains high amounts of PUFAs and provides a balanced ω-6/ω-3 ratio (Sánchez-Machado and others 2004, Mouritsen and others 2013a).

The carbohydrate content in *P. palmata* was found to be around 45% of its dry mass (Morgan and others 1980). Lahaye and others (1993) categorized dulse as a rich source of dietary fiber in the early 90s. Although it is common to find galactans in abundance in red sea vegetables, xylans are the primary polysaccharide constituent in dulse (Morgan and others 1980, Usov 2011). This might explain the absence of dulse from the agar and carrageenan industry. Dulse contains chlorophyll a, water-soluble phycobiliproteins (R-phycocyanin, R-phycoerythrin, allophycocyanin, *β*-phycoerythrin) and carotenoids such as α- and β-carotene, lutein and zeaxanthin (Morgan and others

1980). The loss of red color upon heating can be attributed to the loss of its water-soluble pigments.

Dulse contains biologically active compounds including vitamin C, vitamin E, *β*-carotene, chlorophyll, lutein and various polyphenols. In comparison to nori, it was found to have lesser amounts of bioactive compounds (Ferraces-Casais and others 2012). Although dulse exhibits scavenging activity (Yuan and others 2005a, 2005b), one group of researchers found that its extracts were less effective as antioxidants in comparison to common industrial antioxidants such as ascorbic acid and BHA (Yuan and others 2005a).

1.7.4. *Gracilaria spp.*

With over 100 species in this genus, *Gracilaria spp* belong to Rhodophyta and have been cultivated since mid-1900s, in particular for agar production (Santelices 2014). These fast-growing species grow at warm temperatures, usually around 15 to 25ºC (Yarish and others 2012, Baghel and others 2014, Santelices 2014). Their morphology is distinct compared to other sea vegetables, where the thallus is further branched into round or flattened "stick-like" blades (Fig 1.4). The availability of nutrients, light and salinity affects the pigments and thus the color of the sea vegetable, which is from light red to almost black (Yarish and others 2012, Baghel and others 2014).

Figure1.4. *Gracilaria edulis* frond

Image from Santelices (2014)

G. coronopifolia is a popular species in Hawaii sold fresh under the name *Limu*, which translates to algae. It is added to dishes prepared with fish or meat to add crunch and color (Abbott and others 1978, The University of Hawai'i 2001, Paull and Chen 2008). The only *Gracilaria* species native to New England is *G. tikvahiae* which is cultivated in the sea as well as in tanks (Yarish and others 2012). Although a considerable amount of work has been done on efficiently cultivating *Gracilaria spp.* for agar production, their potential as nutritional food sources remain fairly underexplored.

McDermid and Stuercke (2003) reported moisture content of multiple *Gracilaria spp.* to be 90% on fresh weight basis. The ash content of these species was reported to range widely, from 22.7 to 53.1% of dry mass. The high mineral content in these species has considerable amounts of potassium, sodium, calcium, magnesium and iron compared to land vegetables, and may provide key nutrients to mineral-deficient populations (Norziah and Ching 2000, McDermid and Stuercke 2003, Tabarsa and others 2012b,

Baghel and others 2014). Carbohydrates contribute significantly to *Gracilaria'*s biomass, ranging from ~42.0 to 70.5 % of dry mass.

Following a trend similar to other marine macroalgae, the lipid content in this genus is reported to be below 3% whereas the crude protein ranged from 5.2 to 19.3% (dwb) (McDermid and Stuercke 2003, Hong and others 2007, Rohani-Ghadikolaei and others 2012, Tabarsa and others 2012b, Baghel and others 2014). The fatty acid profiles of these species has shown them to be high in arachidonic acid (Tabarsa and others 2012b, Robertson and others 2013). Within the protein fraction, glutamic and aspartic acids are present in high amounts which impart the unique flavor associated with sea vegetables (Gressler and others 2010, Tabarsa and others 2012b, Baghel and others 2014). Vitamin C content was reported to be 28.5 mg/100g (Norziah and Ching 2000) and 7.3 mg/100g (Hong and others 2007) on a fresh weight basis in *G. changgi* and *G. tenuistipitata*, respectively.

1.8. Research Needs

Prior research has proved that nutritional content of sea vegetables varies among and within species, and is affected by differences in harvest season, location and other environmental factors. Moreover, some studies investigated commercially available dried sea vegetable products whereas others harvested fresh and subsequently dried the sea vegetable for analysis. Most of the studies reported results obtained for wild harvested species. Since aquaculture is developing, more research is needed in this area.

Most of the commercial sea vegetable food products available in the market have been previously processed, usually sun or oven dried. Hence, almost all of the research on sea vegetables has focused on dried sea vegetable products. However, in a recent

article about the top ten food trends in North America, the author cited multiple trend reports showing that almost 9 out of 10 adults consider fresh foods to be healthier and that 78% of consumers try to eat more fresh versus processed foods. Also, while thermal processing can help extend the shelf life of fresh vegetables it may also have undesirable effects on heat sensitive compounds such as vitamin C, therefore, lowering the amount of biologically active compounds. Antioxidant capacity, total phenolic content and amino acid content can be negatively affected by drying and storage (Jiménez-Escrig and others 2001, Wong and Cheung 2001).

Upscale restaurants are moving beyond Asian cuisine to offer innovative fresh sea vegetable dishes to adventurous consumers. However, a lack of information about fresh, farm-raised sea vegetables poses a roadblock to purchasing, storing, and utilizing this unfamiliar product. For instance, the shelf life of fresh sea vegetables is thought to be very short. Although multiple authors have made such claims, the literature on this subject is too scarce to make any judgements. Removing moisture guards sea vegetables from deteriorating and increases their shelf life; making drying a crucial processing step (Wong and Cheung 2001, Gupta and others 2011). A storage study on dulse and *Ulva rigida* (green sea vegetable) stored at 4 ºC (39.2 ºF) assessed mesophilic aerobes, fungi and yeasts for two weeks and found low levels throughout (Liot and others 1993). Paull and Chen (2008) assessed shelf life and different treatment combinations to extend storage life of fresh aquacultured *Gracilaria sp*. They recommended a shelf life of 4 days, based on averaging results from different treatments. However, keeping the samples fully submerged in seawater in the dark extended the shelf life to nearly 30 days. The authors also suggested that the quality loss may have been tied to nitrogen and nitrate content.

Shelf life and quality of fresh fruits and vegetables are dependent on their nutritional composition, storage conditions and handling. Being a highly variable product, proper shelf life analysis on each species of sea vegetable is vital to determine its postharvest life. Extrinsic factors such as geographic origin, season and life cycle likely affect shelf life greatly as well. However, thus far there has been extremely limited investigation in this area, with no reports on refrigerated shelf life of fresh, farm-raised sea vegetables from New England.

1.9. Objectives

The general aim of this research was to generate meaningful data about sea vegetables that could be used for product diversification and marketing purposes by the developing sea vegetable aquaculture industry. Results from this study will provide crucial information on shelf life, basic nutritional composition, total phenolic compounds and antioxidant capacity of fresh and minimally processed dulse, *Gracilaria*, sugar kelp and winged kelp. The specific objectives were as follows:

Objective 1: To determine shelf life of two freshly harvested farm-raised brown sea vegetables (sugar kelp and winged kelp) in two product forms (whole fronds and sea vegetable slaw), and two red sea vegetables (dulse and *Gracilaria* whole fronds) under refrigeration for up to two weeks based on sensory, microbial, physical and biochemical evaluations.

Objective 2: To determine nutritional composition including proximate analyses, selected minerals and vitamin C content of fresh, farm-raised dulse, *Gracilaria*, sugar kelp and winged kelp.

Objective 3: To determine the effects of minimal processing (blanching and freezing) and tissue type (frond and stipe) on phenolic content and antioxidant capacity of two brown (sugar kelp and winged kelp) and two red sea vegetables (dulse and *Gracilaria*).

CHAPTER 2

REFRIGERATED SHELF LIFE AND NUTRITIONAL ANALYSES OF TWO FRESH RED SEA VEGETABLES, DULSE (*Palmaria palmata*) AND *Gracilaria tikvahiea*

2.1. Justification and Objectives

Sea vegetable farmers in New England have started cultivating, developing, and distributing fresh sea vegetables. In addition to dried and/or rehydrated sea vegetables, restaurant owners now have the option to purchase fresh sea vegetables for inclusion in gourmet seafood dishes. Two red sea vegetable varieties, dulse and *Gracilaria*, are among the few species that are currently being wild harvested and farm-raised in the Gulf of Maine. As local Maine producers begin to sell fresh sea vegetables, primarily to upscale regional restaurants, detailed information on their loss of quality during refrigerated storage will lay the foundation for robust distribution of these niche products. Paull and Chen (2008) reported an average shelf life of four days for fresh farm-raised *Gracilaria* from Hawaii. They also determined that light exposure, sea water storage and temperature affected shelf life of this species. There have been no prior reports in the literature on the shelf life of fresh dulse, which continues to gain popularity among American consumers due to its high protein content and palatable flavor (Mouritsen and others 2013). Additionally, nutrient contents of fresh, farm-raised dulse and *Gracilaria* have been scarcely reported.

The overall aims of this study were to estimate the shelf life of freshly harvested dulse and *Gracilaria* at two storage temperatures, 35 ºF and 45 ºF, and to assess their nutrient contents. The lower storage temperature, 35 ºF, is recommended for many fresh

fruits and vegetables (Gast 1991) whereas the higher temperature, 45 ºF, more closely reflects conditions commonly observed in restaurant refrigerators, which are repeatedly opened and closed throughout the day. The specific objective of this study was to assess quality changes of fresh dulse and *Gracilaria* at 35 ºF and 45 ºF for up to two weeks as determined by: 1) sensory evaluation, 2) microbiological assay, 3) physical quality (color, texture, drip loss) and 4) soluble protein content. A second objective was to analyze proximate composition, and to determine vitamin C and selected mineral contents of fresh dulse and *Gracilaria*.

2.2. Materials and Methods

2.2.1. Shelf Life Studies

Two separate shelf life studies were conducted on Dulse (Du), *Palmaria palmata,* (February harvest) and *Gracilaria tikvahiae* (Gr) (September harvest), based on their availability. Effects of two refrigeration temperatures, 35 ºF and 45 ºF, on microbial, sensory and physicochemical quality of samples were assessed every 2-3 days for up to 2 weeks or until samples were unfit for human consumption. Freshly harvested crops from Maine Fresh Sea Farms (Clark Cove, Maine) were cleaned with sea water and packaged into 2-gallon ziploc bags and delivered in coolers on ice the next day for Dulse and the same day for *Gracilaria*.

For Dulse, all analyses except instrumental texture were performed on days 1,3,5,7,9 and 11 of storage. There was no sensory evaluation on day 5 since it was during the weekend and panel members were not available. Instrumental texture was assessed on days 4,6,8,10,12. For *Gracilaria*, all analyses except instrumental texture were performed on days 1,3,5,8,10 and 12. Instrumental texture was assessed on days 2,4,6,9,11 and 13.

Dulse was coded Du 35 or Du 45 for samples stored at 35 ºF and 45 ºF, respectively. For *Gracilaria,* samples stored at 35 ºF were coded Gr 35 whereas samples stored at 45 ºF were coded Gr 45. Triplicate (A, B, C) batches of all treatments were processed and analyzed throughout the study.

2.2.1.1 Sample Preparation

Upon delivery, ~6.5 kg of dulse and ~6 kg of *Gracilaria* fresh samples were divided into twelve 2.5-gallon plastic ziploc bags. There were 2 bags per treatment replicate, with a total of 12 bags for each species. Each bag contained 500 g and 460 g sample for Dulse and *Gracilaria*, respectively. Additionally, one bag with 500 g *Gracilaria* was prepared per treatment replicate to perform drip loss analysis. Any sample with visible biofouling i.e. that appeared to be degraded or contain numerous epiphytes, was removed from the study prior to packaging. The bags were stored in a cooler on ice until all the bags were prepared and then the samples were stored in two separate refrigerators, one held at 35 ºF and another at 45 ºF. The bags were randomly placed on refrigerator racks to reduce any effects due to potential uneven refrigerator temperatures. This day was considered as Day 0 of the shelf life study. For each testing day, the required amount of sample was taken out from each replicate bag in the morning and was stored in coolers on ice while analyses were in process. Plastic trays were used to separate samples from the ice in the cooler to avoid chilling injury. Refrigerator temperature was recorded each testing day and was adjusted as needed to maintain appropriate storage conditions (35 ºF or 45 ºF).

2.2.1.2. Sensory Evaluation

Twelve panelists, 18 years of age or older, who were familiar with seaweed products were recruited from the University of Maine community via word of mouth. Participants interested in sea vegetables and committed to attending a majority of the test days during each 2-week shelf life experiment were included and briefly trained on each species to evaluate specific quality attributes. During the shelf life study, samples were rated based on visual observation, aroma, and touch. The evaluation sheet (Appendix A and B) comprised a 15 cm unstructured line scale for each attribute of interest, with 0 as the poor quality score and 15 as the excellent quality score. Opposite descriptors were attached to either ends of the line scale. The descriptors were developed based on preliminary assessment and discussion with the panel. The panelists were provided with an informed consent form (Appendix C) prior to participation. Approval for research with human subjects was obtained from the Institutional Review Board (IRB) prior to conducting sensory analyses. Each test day a set amount of sample for each treatment (7 g for Dulse and 8 g for *Gracilaria*) was taken out from each replicate bag and pooled together on a white ceramic plate. Each plate was labeled with a 3-digit randomized code for each day. The testing took place under normal white light. Panelists were provided a paper ballot and were encouraged to write comments in the comments section of the evaluation sheet. The recorded ratings were measured using a 15 cm ruler.

2.2.1.3. Aerobic Plate Counts (APC)

Each testing day approximately 16 g of sample were taken out from each treatment replicate to determine aerobic plate counts (APC). Aseptic techniques were employed to place 15 g of sample in a stomacher bag with filter and to add 0.1% sterile

bactopeptone (BD Diagnostics, Sparks, MD) (1:10 w/v). The samples were mechanically mixed for 2 min using a BAGMixer 400 (Model P, SpiralBiotech, Advanced Instruments, Norwood, MA). Initially, one mL aliquots from three serial dilutions (1:10, 1:100 and 1:1000) were plated on Petrifilm™ Aerobic Count Plates (3M, St. Paul, MN). The plates were incubated for 48 h at 37 ºC after which films with 30-300 colonies were counted and recorded. The dilutions were increased as necessary depending on the total plate counts. All the three replications for each treatment were analyzed in duplicate and the values were averaged. To obtain the colony forming units (CFU) of bacteria per gram of sample, the plate count number was multiplied by the dilution factor.

2.2.1.4. Physical Analyses

2.2.1.4.1. Colorimetric Analyses

Colorimetric analyses were performed using a Hunter L*a*b* colorimeter (LabScan XE, Hunter Labs, Reston, VA) in which L* value is based on a scale of dark (0) to light (100), a* value is based on a scale of green (-) to red (+), and b* value is based on a scale of blue (-) to yellow (+). Black and white ceramic standard plates were used to standardize the colorimeter before each use and the colorimeter was allowed to warm up for 30 min prior to color analysis. A port size of 50.5 mm, area view of 44.5 mm, and D65 illumination were used. The disc with 5.1 cm diameter hole was used. Each testing day 90 g of sample was used for obtaining 10 readings in total from each replicate. One layer of sample was spread on the colorimeter glass cup, which was 2.5-inch in diameter, to cover the base of the cup. Each sample was read 3 times by rotating the colorimeter cup 120º after the initial reading, and the values were averaged to provide one reading per sea vegetable sample.

2.2.1.4.2. Texture Analyses

Different anatomies of dulse and *Gracilaria* led to different methods for analyzing their texture. Texture profile analysis was employed for dulse due to its thin, flat blades whereas a Kramer shear force method was developed for *Gracilaria* since the thalli branches were firm and snappy.

2.2.1.4.2.1. Texture Profile Analysis (TPA)

Eighty grams of dulse, were cut into 3 cm x 3 cm squares using a cookie cutter and then stacked to 0.8 cm in height. The texture analyzer (TA-XTi2, Texture Technologies Inc., Scarsdale, NY) was calibrated using a 5,000 g load cell from the same company. A 2-inch diameter cylindrical probe was used with 1 mm/sec pre-test speed, 2 mm/sec test-speed and 2 mm/sec post-test speed and with a distance of 0.3 cm. Force in Newton (N) was recorded by the texture analysis software (Exponent 32, version 5.0, 6.0, 2010, Texture Technologies Inc., Scarsdale, NY). A total of 8 values were averaged per treatment replicate.

2.2.1.4.2.2. Kramer Shear Force

Gracilaria samples were packed 3 cm deep in a mini Kramer shear cell (TA-XTi2, Texture Technologies Inc., Scarsdale, NY). A total of five flat blades were attached to the fixture. The pre-test and test speed was set to 1 mm/sec whereas the post-test speed was set to 10 mm/sec with a distance of 2.9 cm. The texture analyzer (TA-XTi2, Texture Technologies Inc., Scarsdale, NY) was calibrated using a 5,000 g load cell from the same company before each use. Force (N) required to shear the sample was recorded by texture analysis software (Exponent 32, version 5.0, 6.0, 2010, Texture Technologies Inc., Scarsdale, NY). A total of 10 values were averaged and used per treatment replicate.

2.2.1.4.3. Drip Loss

During the shelf life study for *Gracilaria*, drip loss was recorded to assess how much tissue fluids were lost during storage. Drip loss was measured by decanting and weighing all the tissue fluids. On each testing day, the sample bags were tilted for 30 seconds to remove the cellular liquid. Percent loss was calculated based on the initial sample weight and fluid loss of the sample.

$$\% \text{ drip loss} = \frac{\text{fluid loss (g)}}{\text{initial sample weight (g)}} \times 100$$

2.2.1.5. Soluble Protein

Soluble protein was extracted using the methods described by Paull and Chen (2008) with slight modifications. Eight grams of sample were chopped and subsequently homogenized with 32 mL sodium phosphate buffer (pH 7) using a Polytron homogenizer (Brinkmann Instruments, Westbury, NY) for 2 minutes. Homogenized samples were subjected to centrifugation (Beckman J-25, Brea, CA) at 14,000 xg for 15 minutes. The supernatant was collected and frozen at -20 ºC until further analyses. Protein analysis for dulse was performed as described by Lowry (1951). Briefly, 5 mL of freshly prepared solution containing 2% sodium carbonate (Sigma Aldrich, St. Louis, MO) in 0.4% sodium hydroxide (Fisher Scientific, Waltham, MA), 1% cupric sulfate (Fisher Scientific, Waltham, MA) and 2.7% sodium potassium tartrate (Fisher Scientific, Waltham, MA) in a ratio of 100:1:1 was added to 100 µL of sample extract and incubated for 10 min. Another 500 µL of Folin's Ciocalteu Reagent, 2N (Fisher Scientific, Waltham, MA) diluted with distilled water 1:2 was added and incubated for 40 min. One mg/mL Bovine Serum Albumin (BSA) was used in varying volumes (0-200 µL) as a standard. Distilled water

(100 µL) was used as the blank. Absorbance was read at wavelength 700 nm using a UV-vis spectrophotometer (Beckman Du 530, Brea, CA). Protein precipitation was observed during *Gracilaria* protein content analysis following Lowry and others (1951). Consequently, Bradford (1976) was used to assess soluble protein of *Gracilaria* samples. Different concentrations (0-0.3 mg/mL) of BSA were used as the standard, with zero mg/mL used as the blank. Briefly, 5 mL coomassie blue dye was added to 100 µL of sample extract or standard solution and samples were incubated for 30 min. Absorbance was checked at wavelength 595 nm using a UV-vis spectrophotometer (Beckman Du 530, Brea, CA).

2.2.1.6. Statistical Analyses

Data were analyzed using JMP 12.2 (SAS Software, Cary, NC). Shapiro-Wilk's normality test and Levene equality of variances were used to assess data prior to further analyses. Multiway ANOVA was used to assess overall effects of storage time and temperature. One-way analyses of variance (ANOVA) was selected to find treatment differences each day. Tukey's Honest Significant Difference (HSD) test was selected for post-hoc analyses. In cases where data did not satisfy normality, homogeneity or independence, they were transformed logarithmically or by squaring. In cases where transformation failed to satisfy data distribution assumptions, data were analyzed non-parametrically using Kruskall-Wallis. Steel-Dwass test was selected for post-hoc analyses post non-parametric analyses. A significance level of $p<0.05$ was chosen for all statistical analyses.

2.2.2. Nutritional Analyses

2.2.2.1. Sample Preparation

Approximately 500 g of fresh sea vegetables were pureed using a food processor and dried in a convection oven (VWR International, Radnor, PA) at 105 ºC until reaching a constant weight. The dried sample was crushed and ground further by using a motor and pestle. The ground sample was stored in a whirlpack bag in a desiccator until nutritional analysis. Three subsamples for each analysis were used from this homogenous powder. Moisture content and vitamin C content were analyzed on freshly pureed samples.

2.2.2.2. Moisture Content

Moisture content of pureed sea vegetables was determined gravimetrically according to the AOAC method 950.46 by drying 5 g sample in a pre-weighed aluminum pan in triplicate overnight in a convection oven at 105 ºC (VWR International, Radnor, PA) (AOAC 2005). Pans containing the samples were re-weighed and the percent moisture was calculated as follows:

$$\text{g/100g Moisture} = \frac{[\text{pan wt. (g)} + \text{wet sample wt. (g)}] - [\text{pan wt. (g)} + \text{dry sample wt. (g)}]}{\text{wet sample wt. (g)}} \times 100$$

2.2.2.3. Ash Content (Total Minerals)

Ash content was also determined gravimetrically according to the AOAC method 938.08 (AOAC 2005). Two hundred mg of oven-dried sample in a pre-weighed scintillation vial was charred on a hot plate set on medium. The samples were charred until there was no smoke coming out of the samples. The sample vials were then placed in a muffle oven (Thermolyne Model F-A1730, Dubuque, IA) at 550 ºC for six hours.

Vials containing the samples were re-weighed and the percent ash on dry basis (dwb) was calculated as follows:

$$g/100g\ Ash = \frac{[vial\ wt.\ (g) + ash\ wt.\ (g)] - vial\ wt.\ (g)}{dry\ sample\ wt.\ (g)} \times 100$$

2.2.2.3.1. Selected Minerals

The ashed samples in scintillation vials were dissolved with 7 mL of concentrated *omni*trace nitric acid (EM Science, USA) and 1 mL of hydrochloric acid (Fisher Scientific, Waltham, MA). After the bubbling of samples had stopped, approximately 10 mL of distilled water was added and the samples were vortexed for approximately 5 s. The contents of the vial were poured into a 100 mL volumetric flask and brought to volume with distilled water, stirred, and allowed to settle overnight. Approximately 10-15 mL of each sample was poured into a new pre-labelled scintillation vial and then analysed by inductively coupled plasma optical emission spectroscopy (ICP-OES) (Thermo Elemental IRIS Interpid DUO ICP-OES, USA) to determine calcium, potassium, magnesium, phosphorous, aluminum, copper, iron, sodium and zinc. All the samples were analyzed in triplicate.

2.2.2.4. Crude Fat Content

The fat content was determined by the AOAC acid hydrolysis method 948.15 (AOAC 2005). Two and a half grams of oven-dried samples were added to French Square bottles with 10 mL of 8.1 N hydrochloric acid (Fisher Scientific, Waltham, MA) and were placed in a water bath at 85-90 ºC for 90 minutes. The samples were cooled prior to adding 7 mL of ethanol (Fisher Scientific, Waltham, MA) and swirling for 15 s. Twenty-five mL of ethyl ether (Fisher Scientific, Waltham, MA) were added to the sample and shaken for 60 s. For the first 15 s the samples were moderately shaken and then vigorous

shaking followed for 45 s. Following this, twenty-five mL of petroleum ether (Fisher Scientific, Waltham, MA) were added to the sample and shaken for 60 s in the same fashion. The samples were then allowed to settle for at least 30 min to allow the emulsion to break. The top layer (ether plus fat) was carefully extracted using a glass pipette and transferred to a pre-weighed flat bottom beaker. Three more extractions were performed using 15 mL of ethyl ether and petroleum ether followed by shaking and adding the top layer to the previously collected pool. The pooled ether with lipid was allowed to dry overnight under the chemical hood followed by drying in a 105 ºC the oven for 7 minutes. The fat content (dwb) was calculated by reweighing the cooled beakers and using the following formula:

$$\text{g/100g Crude Fat} = \frac{[(\text{flask (g)} + \text{fat weight (g)}) - \text{flask weight (g)}]}{\text{sample weight (g)}} \times 100$$

2.2.2.5. Crude Protein Content

The nitrogen content of the dried samples was determined by combustion analyzer (TRU MAC CNS, LECO Corp., MI, USA) using oven-dried samples. The crude protein content (dwb) was calculated by multiplying the nitrogen content by a conversion factor of 6.25 used for seafood. Each analysis was performed in duplicate.

2.2.2.6. Carbohydrate Content

The carbohydrate content of the samples was calculated by difference as follows:

g/100g Carbohydrate = 100 - (ash content + fat content + protein content)

2.2.2.7. Vitamin C

Vitamin C was determined according to AOAC methods 967.21 and 985.33 by titrating sample extracts using 2,6-dichlorophenolindophenol dye (AOAC 2005). Eight grams of fresh pureed sample were homogenized with 15 mL precipitant solution using a

Polytron homogenizer (Brinkman Instruments, Westbury, NY) for 2 min and centrifuged (Beckman J-25, Brea, CA) at 10,000 xg for 15 min at 4 ºC. The pellet was re-suspended in 15 mL precipitant solution and centrifuged again. The supernatants were pooled together and the final volume was recorded. The precipitant solution was made by mixing equal amounts of two solutions. The first solution was made by dissolving 15 g of glacial meta-phosphoric acid in 40 mL glacial acetic acid and bringing it to 250 mL with distilled water. The solution was filtered using a P8 qualitative paper (Fisher Scientific, Waltham, MA). The second solution was made by dissolving 0.9 g ethylene diamine tetra acetic acid (EDTA) (Sigma Aldrich, St. Lois, MO) in 200 mL of distilled water and bringing it up to 250 mL. The precipitant solution was made fresh on the day of use. Ascorbic acid (1 mg/mL) was used as the standard solution and was prepared fresh by diluting 50 mg ascorbic acid (Fisher Scientific, Waltham, MA) to 50 mL with the precipitant solution in a volumetric flask. For the dye, 0.0625 g of 2,6-dichlorophenolindophenol sodium salt and 0.0525 g of sodium bicarbonate (Fisher Scientific, Waltham, MA) were brought up to 250 mL with distilled water. After mixing thoroughly, the solution was passed through a fisher P8 filter (Fisher Scientific, Waltham, MA). The ascorbic acid standard plus 5 mL precipitant solution was titrated using the indophenol dye until rose pink color persisted for 10 s. Fifteen mL aliquots of sample extracts were poured in 50 mL Erlenmeyer flasks and titrated with the indophenol dye until the rose-pink endpoint lasted for 10 s. For the sample blank, two 15 mL aliquots of precipitant solution were added into separate 50 mL Erlenmeyer flasks and titrated with indophenol standard solution to obtain the same endpoint. The ascorbic acid concentration of the sample was calculated using the following formula:

mg of ascorbic acid/g or mL of sample = C x V x (DF/WT)

where, C = mg of ascorbic acid/mL of dye,

V = mL of dye used for titration of diluted sample (subtract blank volume first),

DF = dilution factor and WT = sample weight (g).

2.2.2.8. Data Presentation

Analytical replicates were averaged (± standard error) and reported on a dry weight basis, except for moisture and vitamin C content. The macronutrients were presented in g/100g whereas the micronutrients and vitamin C were presented in mg/100g.

2.3. Results and Discussion

The two shelf life studies on the red sea vegetables offered information that will be extremely beneficial to ongoing sea vegetable research. Even though both the species analyzed were red sea vegetables, there were obvious anatomical differences between them. Dulse had flat, blade-like fronds whereas *Gracilaria* had thick stick-like branches. Their color differed as well, with *Gracilaria* having extremely intense red color compared to the much lighter dulse. As dulse wilted, the samples clumped together and gave an evident off-odor. The aroma/off-odor was more intense when the storage bag was first opened each testing day compared to when the samples were taken out and presented to the sensory panelists later in the day.

2.3.1. Shelf Life Studies

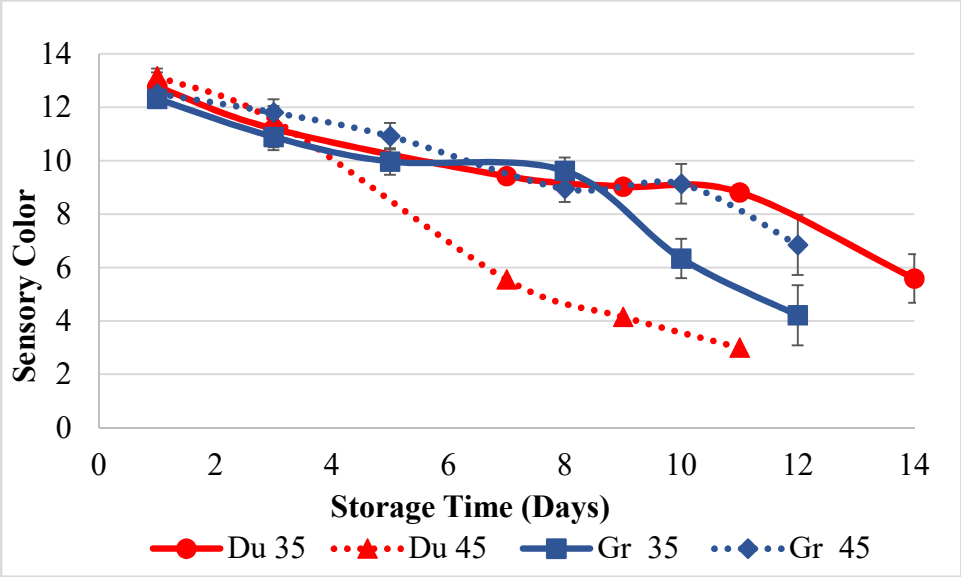
2.3.1.1. Sensory Evaluation

A 15 cm scale was used with opposite descriptors at either ends to rate sensory color, aroma, texture and overall quality (Appendix A, B). These descriptors were

determined during the training sessions based on panelists' suggestions and agreement. The best quality score was 15 and the lowest quality score was 0. For dulse color, the descriptors went from dark plum-red (score 15) to faded plum-red (score 0) whereas for *Gracilaria* color, the descriptors ranged from dark brown-red (15) to faded brown-red (0). For aroma, the descriptors used were pleasant (15) and unpleasant (0) for both the species. For dulse, sheen was assessed as an attribute using descriptors dull (0) and glossy (15). For dulse texture, the the scale ranged from strong (15) to fragile (0). For *Gracilaria* texture, these descriptors were firm (15) versus limp (0). Overall quality was assessed with fresh (15) and complete loss of freshness (0) as descriptors.

The sensory color scores for dulse and *Gracilaria* samples were significantly ($p<0.05$) affected by time and temperature. However, a combined effect of time and temperature was observed only for dulse samples. On a scale from 0 to 15, both, Du 35 and Du 45 had scores of approximately 13 on day 1 but the scores for Du 45 fell significantly to 5.5 by day 7 (Fig 2.1). However, for Du 35, the scores did not drop significantly until day 14, indicating that the samples faded faster at the higher storage temperature. For *Gracilaria* samples, the scores (~10) for its attractive red color were similar for both the temperature treatments until day 8. However, the sensory scores dropped to ~6 on day 10 for Gr 35 while samples stored at the higher temperature continued to receive higher scores (~10). It was observed that the samples, especially *Gracilaria,* did not lose color in any particular spatial pattern. Discolored patches on a frond or thalli randomly appeared and made it difficult to assess color consistently. Similar findings were reported by Paull and Chen (2008), who discussed that even within *Gracilaria* clusters, the discoloration was not uniform.

Figure 2.1. Sensory color scores for Dulse and *Gracilaria* during refrigerated storage.



Each value represents the mean ± standard error, (n=8-12, depending on test day).

The panelists were asked to sniff the samples briefly and rate the aroma. Time, temperature and their interaction had a significant effect on sensory aroma of dulse samples but surprisingly, only time affected sensory aroma of *Gracilaria* samples (Table 2.1). The lower storage temperature maintained "pleasant" aroma in dulse for a longer time compared to the higher temperature, where the scores dropped significantly by day 7 compared to day 1. Some panelists mentioned that they could smell a "fishy" aroma in Du 45 by the end of the study. Although the aroma scores dropped significantly by day 8 for Gr 45 compared to day 10 for Gr 35, the former treatment maintained its score at approximately 7 until the end of the study.

Table 2.1. Sensory aroma values during refrigerated storage.

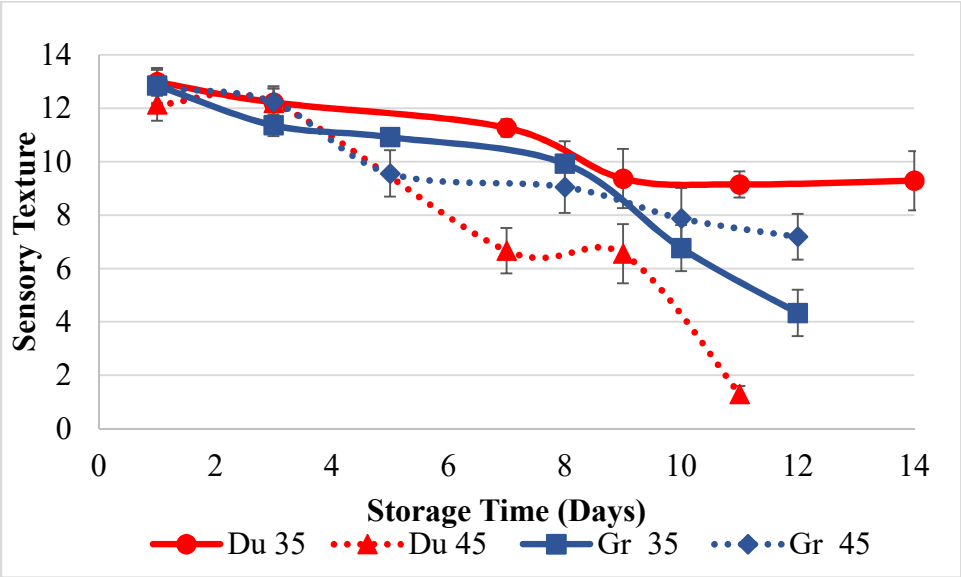
Dulse			*Gracilaria*		
Day	**Du 35**	**Du 45**	**Day**	**Gr 35**	**Gr 45**
1	12.9 ± 0.5b	13.2 ± 0.2b	1	12.3 ± 0.4c	12.4 ± 0.5b
3	10.8 ± 0.9b	10.9 ± 1.2b	3	11.4 ± 0.8c	11.9 ± 0.5b
7	9.5 ± 0.9b	6.2 ± 0.8a	5	10.0 ± 0.7bc	9.9 ± 0.7ab
9	9.7 ± 0.9b	5.4 ± 1.3a	8	9.9 ± 1.0bc	7.6 ± 1.0a
11	9.1 ± 0.5ab	4.6 ± 0.8a	10	7.2 ± 1.1ab	7.3 ± 1.2a
14	5.0 ± 1.3a		12	4.6 ± 1.2a	7.2 ± 1.1a

Each value is the mean ± standard error, (n=8-12, depending on test day). Values not sharing a letter are significantly ($p<0.05$) different within columns, analyzed by ANOVA followed by Tukey's HSD post hoc test or analyzed non-parametrically by Kruskall-Wallis followed by Steel-Dwass post hoc test.

Panelists were asked to tear the dulse samples whereas they snapped the *Gracilaria* samples to rate the strength and firmness, respectively. Time, temperature and the two combined significantly affected ($p<0.0001$) dulse sensory texture scores, with scores dropping over time but faster for Du 45. Although all the samples scored approximately 13 on day 1 on a scale of 0-15, Du 45 scores plummeted to 6.2 by day 7 whereas, in comparison, Du 35 scores did not fall below 9 throughout storage (Fig 2.2). On day 11, Du 35 scored 9 times higher than Du 45, indicating that 35 ºF maintained the strong texture better than 45 ºF for dulse. Towards the end of the study, panelists mentioned words such as "wilted" and "mushy" to describe the dulse texture. Only time was found to significantly affect sensory texture scores for *Gracilaria.* The scores dropped significantly over time, with samples at 45 ºF scoring approximately 1.5 times higher than those at 35 ºF on day 12. It is noteworthy that even though both the species are red sea vegetables, larger differences in texture values due to temperature were observed in dulse compared to *Gracilaria.* Both the species have completely different physical structure and hence different descriptors were used for their sensory texture.

Some panelists mentioned that towards the end of the study, some *Gracilaria* branches would snap but some did not, indicating that the texture was deteriorating at random spots.

Figure 2.2. Sensory texture scores for Dulse and *Gracilaria* during refrigerated storage.



Each value represents the mean ± standard error, (n=8-12, depending on test day).

For dulse, sheen was also assessed during sensory evaluation. A similar trend was observed that samples stored at 35 ºF scored higher compared to samples stored at 45 ºF (Table 2.2). Over time, the values decreased for both the treatments, however, on day 11, Du 45 sheen scores dropped to 3.38 whereas Du 35 received 8.7. It appeared that sheen was not an appropriate sensory attribute to measure. It was observed that dulse samples did not have much "glossy" sheen in comparison to some other seaweeds and appeared to dry quickly as they were kept out of their packaging.

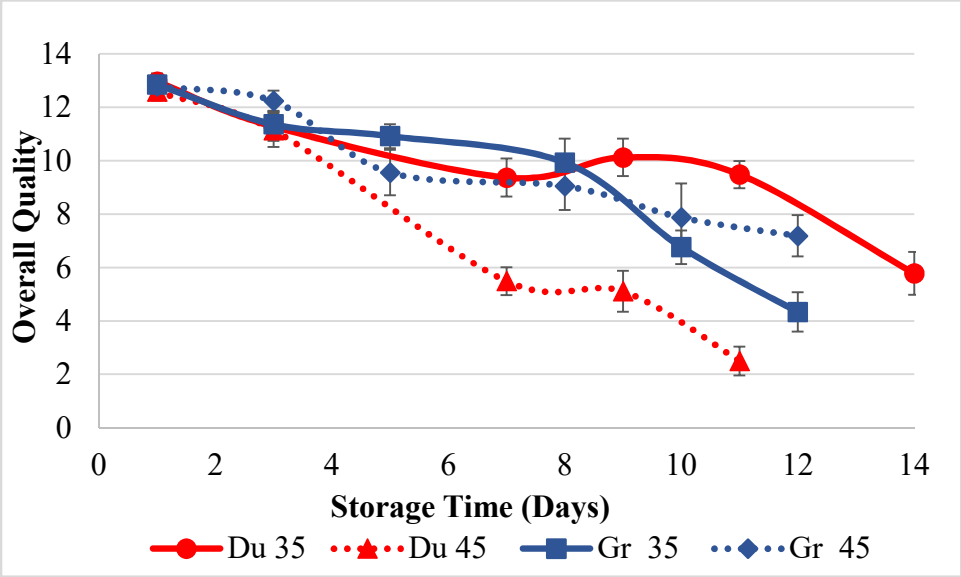
Table 2.2. Sensory sheen values in dulse during refrigerated storage.

	Dulse	
Day	**Du 35**	**Du 45**
1	12.1 ± 0.7c	10.0 ± 0.8c
3	8.7 ± 1.2bc	8.0 ± 1.3bc
7	7.5 ± 1.0ab	5.6 ± 0.9ab
9	9.9 ± 1.0bc	5.1 ± 0.6ab
11	8.7 ± 0.6bc	3.4 ± 0.8a
14	4.4 ± 0.7a	

Each value is the mean ± standard error, (n=8-12, depending on test day). Values not sharing a letter are significantly ($p<0.05$) different within columns, analyzed by ANOVA followed by Tukey's HSD post hoc test or analyzed non-parametrically by Kruskall-Wallis followed by Steel-Dwass post hoc test.

For overall quality, temperature had a large impact on dulse samples whereas the effects were minimal for *Gracilaria* (Fig 2.3), clearly indicating that overall quality was maintained better at lower temperature for dulse samples. This is in agreement with the trend seen for other quality attributes for dulse during storage. Panelists commented that they would not consume Du 45 but would consume Du 35 by day 11. The scores dropped significantly by day 7 for both, Du 35 and Du 45. However, scores dropped below 6 for Du 45 but only to 9.6 for Du 35. Differences between treatments started to appear towards the end of the study for *Gracilaria*, with samples at 45 ºF scoring higher, however, temperature did not have a significant effect on its overall quality scores. These results clearly indicate that the effect of treatment varies between species.

Figure 2.3. Sensory overall quality scores for Dulse and *Gracilaria* during refrigerated storage.

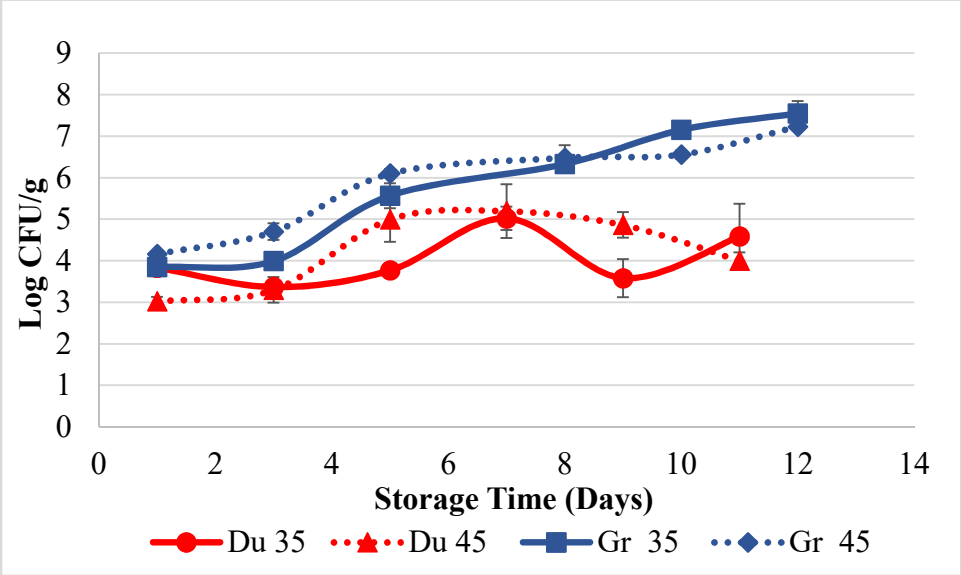


Each value represents the mean ± standard error, (n=8-12, depending on test day).

2.3.1.2. Aerobic Plate Counts (APC)

Microbial spoilage in fresh fruits and vegetables has been studied extensively, since it is linked to loss of quality and safety in foodstuffs. Although there is no critical cut off value for microbial counts in fresh sea vegetables, increased microbial activity over time in fresh fruits and vegetables can lead to lower sensory quality including the production of fermented aromas and affecting acceptability among consumers (Barth and others 2010). Postharvest microbial counts vary depending on various environmental and handling procedures. However, eight out of ten commercially available fresh cut spinach brands contained 7 to 8 log colony forming units (CFU) per gram sample of aerobic bacteria (Abadias and others 2008). Another study reported average aerobic bacterial counts of 6.4 log CFU/g in minimally processed fresh vegetables (Jeddi and others 2014). Debevere (1996) recommended an upper microbial limit of 8 log CFU/g for fresh fruits and vegetables intended for human consumption.

Figure 2.4. Aerobic plate counts of dulse and *Gracilaria* during refrigerated storage.



Each value represents the mean ± standard error, (n=3).

Storage temperature had no significant effect on APC of either species. However, time significantly affected *Gracilaria* samples, which exhibited a steady increase in APC, ranging from 3.8 on day 1 to 7.5 log CFU/g on day 12 (Fig 2.4). Overall, *Gracilaria* samples had higher microbial counts than dulse samples, which ranged from 3.0 at the beginning of the storage to 5.2 log by day 11. One of the reasons for this difference could be their different growing and harvest seasons. Dulse was harvested in winter (February) whereas *Gracilaria* was harvested in late summer (September). The warmer water temperature in the summer, which is closer to the optimal growth temperature of 20 ºC to 45 ºC for mesophilic aerobic bacteria, could have resulted in higher microbial counts for *Gracilaria*. Additionally, different morphologies of these two red sea vegetables may also have impacted microbial growth. Moreover, sea vegetables are known to have several anti-microbial compounds and their extracts have repeatedly shown antimicrobial activity (Cox and others 2010, Gupta and others 2010). This could have aided in keeping the microbial activity below 8 log CFU/g over time. Overall, microbial activity did not

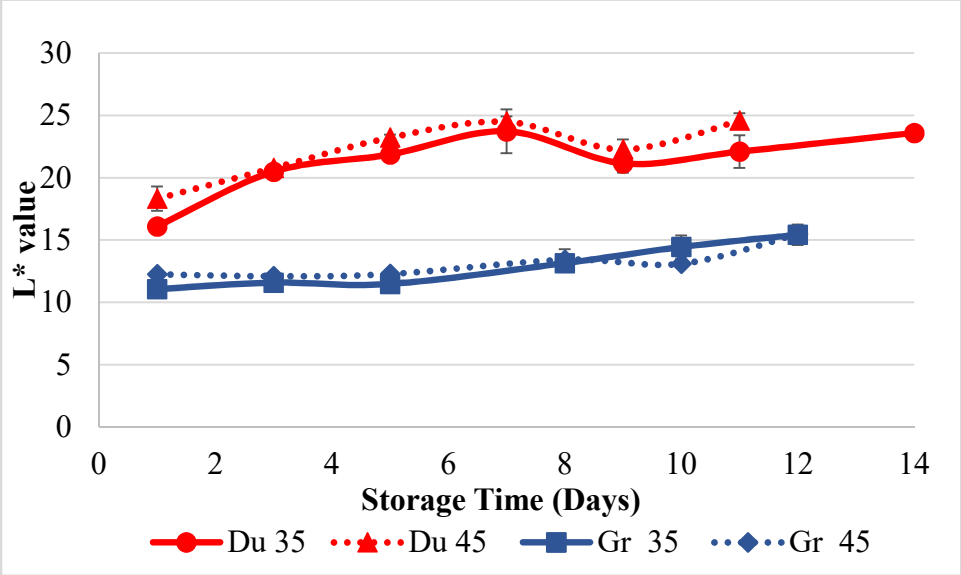
appear to be the primary contributor to quality loss over time for dulse. Although *Gracilaria* microbial counts increased over time, they were below 8 log CFU/g until day 12. Paull and Chen (2008) also concluded that microbial growth was not the primary cause of quality loss based on their low aerobic plate counts over time.

2.2.1.3. Physical Analysis

2.2.1.3.1. Colorimetric Analyses

Colometric L* values are used to measure lightness and range from 0 to 100, where 0 is black and 100 is white. Time and temperature affected L* values of dulse samples significantly, however, only time affected *Gracilaria* samples. The L* increased significantly by day 5 for Du 45 compared to day 1, indicating that dulse samples had faded (Fig 2.5). The L* values did not increase over time until day 10 for Gr 35 and day 12 for Gr 45, indicating that higher temperature maintained the color slightly better for *Gracilaria* compared to lower temperature. The increased lightness, or fading, over time for both the species may be attributed to loss of certain pigments, in particular, water soluble pigments present in red sea vegetables.

Figure 2.5. L* values for dulse and *Gracilaria* during refrigerated storage.



Each value represents the mean ± standard error, (n=3).

The a* values are a measure of redness and were significantly affected by temperature and time for both species. Interestingly, the a* values increased significantly over time for all treatments (Table 2.3), which did not match the fading of red color that was perceived by the sensory panel. However, it is noteworthy that for Du 35 samples the a* values were lower than day 1 through day 11. The clumping of wilted dulse by the end of the study affected the measurement of a* value, with an increase in values on day 14, which could be explained as an artifact effect of the wilted and shriveled dulse samples. On the other hand, *Gracilaria* samples exhibited a gradual increase in redness values over time, which was contrary to the visual fading observed during the course of the study.

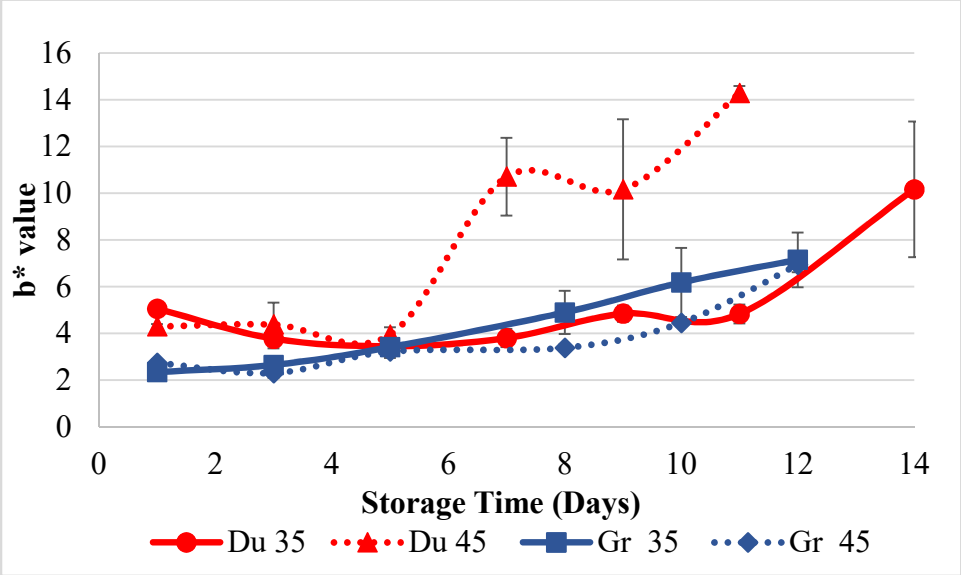
Table 2.3. a* values for dulse and *Gracilaria* during refrigerated storage.

Dulse			*Gracilaria*		
Day	**Du 35**	**Du 45**	**Day**	**Gr 35**	**Gr 45**
1	5.5 ± 0.1a	4.9 ± 0.1a	1	2.5 ± 0.9a	2.5 ± 0.2a
3	3.3 ± 0.3a	3.8 ± 0.7a	3	2.7 ± 0.3ab	2.1 ± 0.1a
5	2.9 ± 0.2a	3.4 ± 0.2a	5	3.4 ± 0.6abc	2.7 ± 0.2a
7	3.4 ± 0.2a	8.0 ± 1.2ab	8	5.5 ± 1.3bcd	3.2 ± 0.3ab
9	3.9 ± 0.3a	7.5 ± 2.1ab	10	7.5 ± 2.4cd	4.4 ± 0.2b
11	4.3 ± 0.4a	10.0 ± 0.2b	12	8.1 ± 1.8d	7.3 ± 0.5c
14	7.0 ± 1.9a	n.d		n.d	n.d

Each value is the mean ± standard error, (n=3). Values not sharing a letter are significantly ($p<0.05$) different within columns, analyzed by ANOVA followed by Tukey's HSD post hoc test or analyzed non-parametrically by Kruskall-Wallis followed by Steel-Dwass post hoc test. n.d= not determined

The b* values measure yellowness of the samples. Time and higher temperature significantly ($p<0.0001$) increased dulse b* values however only time affected *Gracilaria* b* values. b* values were 2.5 times higher for Du 45 compared to Du 35 on day 7 (Fig 2.6). The increased yellowness over time, indicates possible loss of water soluble pigments including phycocyanin and phycoerythrin that contribute to the attractive red color in these sea vegetables (Paull and Chen 2008, Bocanegra and others 2009). Several xanthophylls found in red sea vegetables (Bocanegra and others 2009) that contribute to the yellow color were more visible as the red pigments were lost. Increase in b* values over time in *Gracilaria* has been reported by Paull and Chen (2008) and attributed to physiological changes along with cellular damage, in particular for the lower temperature treatment (<59 ºF). However, the storage temperature in the current study was much lower than that used for the aforementioned study.

Figure 2.6. b* values for dulse and *Gracilaria* during refrigerated storage.



Each value represents the mean ± standard error, (n=3).

In conclusion, measuring L*, a* and b* values over time proved to be useful in assessing quality loss due to fading. However, there were some difficulties faced during these measurements. The change in texture of dulse samples over time resulted in the addition of more sample mass to cover the base of the colorimetry cup. This wilting affected the sample reflectance considerably. Another issue was that the samples faded in random spots and the color values were affected by which part of the frond/thalli was used for the measurement. However, taking an average of 10 measurements for each treatment replicate seemed to tackle this issue to some extent. Measuring color of pureed or homogenized samples instead of intact pieces may result in a more consistent color measurement. However, the objective of this study was to assess surface color deterioration over time.

2.2.1.3.2. Texture Analyses

Overall, TPA force values were significantly affected by time and temperature ($p<0.0001$) for dulse, with the values decreasing over time and with higher temperature. The lower force values towards the end of the study indicate decreased resistance of the samples to compression, which corresponded with the wilting that was observed over time (Table 2.4). On day 12, force values for Du 35 were over 6 times higher than for Du 45. The lower temperature maintained initial dulse texture better than the higher temperature, indicating that the dulse fronds softened more quickly at the higher temperature, which is consistent with the results obtained for sensory texture analyses.

Table 2.4. TPA force and for dulse and Kramer shear force for *Gracilaria* during refrigerated storage.

Dulse			*Gracilaria*		
Force (N)			**Force (N)**		
Day	**Du 35**	**Du 45**	**Day**	**Gr 35**	**Gr 45**
4	190.9 ± 14.3a	123.8 ± 1.1b	2	183.4 ± 8.5a	216.2 ± 23.4a
6	95.2 ± 14.5ab	80.0 ± 13.4b	4	180.8 ± 13.6a	221.2 ± 19.5a
8	118.2 ± 25.3ab	27.1 ± 13.4a	6	206.8 ± 8.2ab	218.3 ± 32.4a
10	103.2 ± 37.6ab	9.6 ± 3.1a	9	243.6 ± 6.0bc	213.6 ± 7.2a
12	48.9 ± 10.9b	7.6 ± 0.5a	11	262.9 ± 27.9c	200.2 ± 16.0a
			13	212.6 ± 4.1ab	184.5 ± 11.4a

Each value is the mean ± standard error, (n=3). Values not sharing a letter are significantly ($p<0.05$) different within columns, analyzed by ANOVA followed by Tukey's HSD post hoc test or analyzed non-parametrically by Kruskall-Wallis followed by Steel-Dwass post hoc test.

Unlike dulse, Kramer shear force values of *Gracilaria* samples were not affected by time or temperature, however, a combined effect of time and temperature was observed. Firmness of Gr 35 increased significantly over time. In contrast to Gr 35, shear force values for Gr 45 did not change significantly. However, the values were decreased

by the end of the study compared to day 1. This could be due to variability among *Gracilaria* samples. Paull and Chen (2008) also assessed *Gracilaria* texture during storage using Kramer shear force method and reported that although the samples became limp over time the data were highly variable.

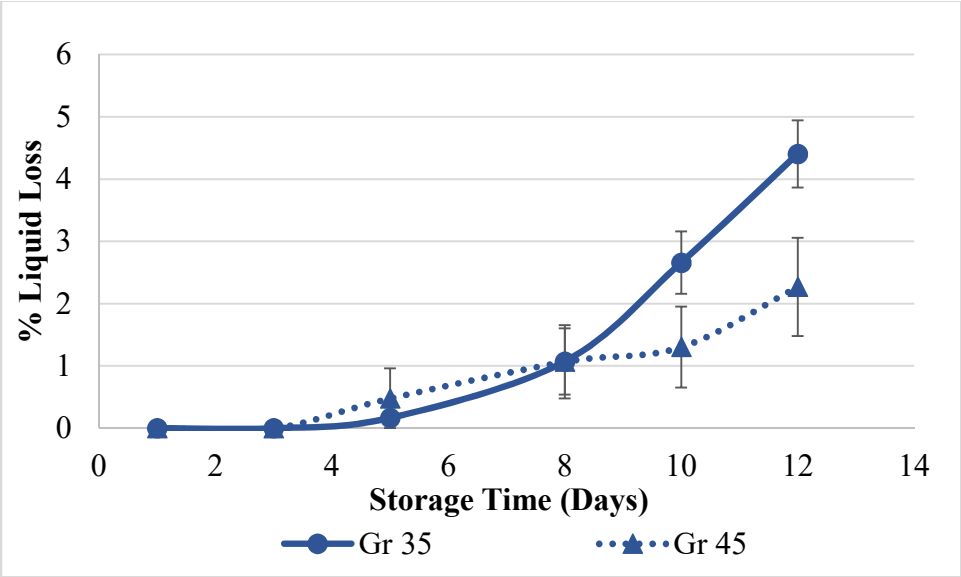
The instrumental texture results were highly variable, with large standard errors. Although the texture visibly deteriorated for both the species, the methods used were not as responsive as the sensory texture evaluation results. Nonetheless, instrumental texture is an extremely crucial parameter to assess quality loss in foods. In the current study, sample height was standardized for both, TPA and Kramer shear force methods. A standardized sample height was chosen due to the obvious wilting of the samples over time. However, standardizing the mass may result in data with lower variability. Moreover, alternate methods other than TPA and Kramer shear could be used to determine changes in texture over time.

2.2.1.3.3. Drip Loss

During the dulse shelf life study, profuse loss of cellular liquid was observed over time as liquid pooled in the bottom of the sample bags. Based on that observation, drip loss was assessed in the subsequent *Gracilaria* study. There was a significant ($p<0.0001$) effect of time, with drip loss accelerating from day 5 onward for *Gracilaria* stored at both the temperatures (Fig 2.7). However, loss of cellular fluid for Gr 35 on day 12 was significantly ($p<0.05$) higher than for Gr 45. This suggests that cellular integrity of *Gracilaria* was maintained better at higher temperature, which was unexpected, but which supports our other findings. Electrolyte leakage, chilling injury and reduced cellular integrity in *Gracilaria spp.* were observed over time for samples stored below

59 ºF (Paull and Chen 2008). Although the storage temperature used in the study discussed was higher than in the current study, it is important to note that the *Gracilaria* samples in the aforementioned study were grown in Hawaii, where the climate is tropical in comparison to the temperate Maine climate.

Figure 2.7. Drip loss values for *Gracilaria* during refrigerated storage.



Each value represents the mean ± standard error, (n=3). Student t-test was performed to determine significant ($p<0.05$) differences between treatments on each testing day.

2.2.1.4. Soluble Protein

No significant differences in the amount of soluble protein were observed over time in either species. Additionally, no significant effects of temperature were seen in either species (Table 2.5). Total protein content, discussed in detail in a later section, in dulse (22 g/100g dwb) was higher than *Gracilaria* (17.8 g/100g dwb), which could be related to the higher values obtained for dulse soluble protein content compared to *Gracilaria.* Additionally, soluble protein was measured following Lowry and others (1951) for dulse and Bradford (1976) for *Gracilaria* since the former method precipitated proteins in *Gracilaria* samples. The different methods used likely played a role in the

differences observed between species. Loss of soluble protein could not be tied to quality changes in dulse or *Gracilaria* in this study. However, Paull and Chen (2008) reported loss of as much as 50% soluble protein by day 6 compared to day 1 and tied it to quality loss. These differences could be due to the high storage temperatures used in their study, 61 ºF and 70 ºF, compared to 35 ºF and 45 ºF used in the current study.

Table 2.5. Soluble protein (mg/g wet weight) values for dulse and *Gracilaria* during refrigerated storage.

Dulse			*Gracilaria*		
Day	**Du 35**	**Du 45**	**Day**	**Gr 35**	**Gr 45**
1	2.2 ± 0.2a	2.0 ± 0.6a	1	0.01 ± 0.00a	0.01 ± 0.00a
3	3.3 ± 0.8a	3.1 ± 1.0a	3	0.01 ± 0.00a	0.01 ± 0.00a
5	2.4 ± 0.4a	3.4 ± 0.7a	5	0.01 ± 0.00a	0.01 ± 0.00a
7	1.6 ± 0.6a	1.9 ± 1.1a	8	0.01 ± 0.00a	0.01 ± 0.00a
9	1.3 ± 0.3a	2.1 ± 0.3a	10	0.01 ± 0.00a	0.01 ± 0.00a
11	1.6 ± 0.3a	2.4 ± 0.6a	12	0.01 ± 0.00a	0.01 ± 0.01a
14	2.2 ± 0.7a	n.d		n.d	n.d

Each value is the mean ± standard error, (n=3). Values not sharing a letter are significantly ($p<0.05$) different within columns, analyzed by ANOVA or analyzed non-parametrically by Kruskall-Wallis. n.d=not determined

2.3.2. Nutritional Analyses

The moisture content of both the red sea vegetables was approximately 90 g/100g (Table 2.6). These values are similar to those previously reported for moisture content of red sea vegetables (McDermid and Stuercke 2003, Bocanegra and others 2009). McDermid and Stuercke (2003) reported moisture content of three *Gracilaria spp.* to be approximately 89.4-90.4 g/100g, which is in agreement with current findings. The ash content of red sea vegetables usually varies anywhere from 22.7 g/100g to 53.4 g/100g dry mass (Morgan and others 1980, McDermid and Stuercke 2003, Baghel and others 2014). Several studies reported high ash content in *Gracilaria spp.*, with the highest

values found in samples from Hawaii (McDermid and Stuercke 2003). On the contrary, the ash values in dulse typically range between 11.7 g/100 to 36.6 g/100 g but in the current study was found to be approximately 44 g/100g (Morgan and others 1980). Seasonal and regional differences may have affected the ash content of dulse. The fat content of dulse and *Gracilaria* was found to be approximately 2 g/100g, well within the 1-4 g/100g range for previously reported fat content of sea vegetables (Morgan and others 1980, McDermid and Stuercke 2003, Hong and others 2007, Rohani-Ghadikolaei and others 2012, Mouritsen and others 2013).

Table 2.6. Proximate composition of fresh dulse and *Gracilaria* (g/100g, dwb) unless specified otherwise.

Species	Moisture (wwb)	Ash	Fat	Protein	Carbohydrate (by difference)
Dulse	90.9 ± 0.1	44.9 ± 0.9	2.0 ± 0.0	22.1 ± 0.0	31.0
Gracilaria	90.1 ± 0.2	44.4 ± 0.3	2.0 ± 0.2	17.8 ± 0.1	35.8

Each value represents the mean ± standard deviation of pooled samples, analyzed in triplicate, except for protein which was analyzed in duplicate.

Red sea vegetables are popular for their high protein content in comparison to brown sea vegetables. Multiple studies have reported protein content of red sea vegetables ranging from 10-47 g/100g (Mabeau and Fleurence 1993, Galland-Irmouli and others 1999, Burtin 2003). The protein content of dulse harvested in the winter was reported be 21.9 g/100g (dwb) (Galland-Irmouli and others 1999), which is extremely close to the protein content of dulse in this study. Other researchers (McDermid and Stuercke 2003, Hong and others 2007, Tabarsa and others 2012) reported protein content approximately 10 g/100g for various *Gracilaria* species. However, this study found higher protein content (17.8 g/100g) for *Gracilaria.* The carbohydrate content was calculated by subtracting the average values for other major food constituents and hence,

does not have standard deviation. Variable carbohydrate content has been previously reported for dulse and *Gracilaria*. MacArtain and others (2007) reported total carbohydrate of dulse 10.6 g/100g (wwb) compared to 2.8 g/100 g (wwb) found in this study. Carbohydrate content of *Gracilaria* from Vietnam was reported to be 70.5 g/100g (dwb) (Hong and others 2007), which was much higher than 35.8 g/100g (dwb), found in this study.

Overall, both the species contained minerals commonly reported in sea vegetables (MacArtain and others 2007, Rao and others 2007, Astorga-España and others 2015). Dulse had higher contents of sodium, phosphorus, aluminum and iron compared to *Gracilaria* (Table 2.7). In both the red sea vegetables, potassium was the most abundant micronutrient whereas copper was not detected. Interestingly, even though sodium and potassium appear to be in high quantities, the Na/K ratio was 0.12 and 0.08 for dulse and *Gracilaria*, respectively. These were extremely low compared to Na/K ratio in vegetable broth (15) and olives (81) (USDA 2014). Low Na/K (<1.5) ratios in sea vegetables have been previously reported by others (MacArtain and others 2007, Rao and others 2007), who explained that consumers suffering from hypertension may enjoy sea vegetables.

Table 2.7. Selected minerals of fresh dulse and *Gracilaria* (mg/100g, dwb)

Selected Mineral	Dulse	*Gracilaria*
Calcium	119.6 ± 8.7	233.1 ± 9.8
Potassium	18,604.4 ± 482.9	19,032.4 ± 288.3
Magnesium	216.4 ± 8.7	270.0 ± 5.8
Phosphorus	323.1 ± 5.3	211.3 ± 4.2
Aluminum	30.2 ± 2.8	25.3 ± 2.5
Iron	50.8 ± 3.6	32.8 ± 3.6
Sodium	2,278.4 ± 46.5	1,537.4 ± 48.9
Zinc	0.2 ± 0.3	2.4 ± 1.6
Copper	0.0 ± 0.0	0.0 ± 0.0

Each value represents the mean ± standard deviation of pooled samples, analyzed in triplicate.

Another micronutrient assessed in this study was vitamin C. The average vitamin C content of dulse and *Gracilaria* was 22.1 ± 0.3 and 1.5 ± 0.1 mg/100g fresh sample, respectively. A study assessing sea vegetable bioactive compounds reported vitamin C content of dulse to be 0.6 ± 0.02 mg/100g fresh sample, which is extremely low compared to the results of this study (Ferraces-Casais and others 2012). However, Morgan and others (1980) reviewed several papers and reported the vitamin C content in dulse to range from 17 to 52 mg/100g fresh weight. Norziah and Ching (2000) reported vitamin C content of *G. changgi* to be 28.5 mg/100g, which is higher than what this study found. However, Hong and others (2007) reported 7.3 mg/100g of vitamin C in fresh *G. tenuistipitata*. Variability and comparatively low vitamin C content of sea vegetables could be a result of differences in species, season and location or due to differences in methods used to determine vitamin C content.

2.4. Conclusions

The results of this study indicate that based on sensory evaluation, an 11-day shelf life for acceptable quality was achieved for dulse samples stored at 35 ºF. For *Gracilaria*, a 10-day acceptable quality shelf life was achieved for samples stored at 45 ºF.

Temperature played a key role in quality loss over time, with the higher temperature reducing the shelf life to 5 days for dulse. Dulse and *Gracilaria* followed opposite trends with regard to temperature effects on quality. Loss of cellular integrity causing drip loss was the leading cause of quality loss over time in both species. Additionally, loss of color also contributed to quality loss. Microbial spoilage did not appear to be a major contributor to quality deterioration. These results are limited to the species and storage conditions used in this study. Prior work in this area is extremely limited, and this study provides a good foundation for ongoing shelf life research. Moreover, these results provide the emerging sea vegetable industry with critical information about the shelf life of fresh red sea vegetables.

Both the red sea vegetables were high in total minerals such as potassium, magnesium and phosphorus, but low in lipid content. Both the species have the potential to supplement mineral deficient diets. They also had considerable amounts of protein, providing an excellent source of amino acids, especially for vegan consumers. The vitamin C content was higher in dulse compared to *Gracilaria*, making dulse attractive to consumers looking for sea vegetables high in certain vitamins. Overall, both the red sea vegetables were nutrient-rich.

CHAPTER 3

REFRIGERATED SHELF LIFE AND NUTRITIONAL ANALYSES OF TWO FRESH BROWN SEA VEGETABLES, SUGAR KELP (*Saccharina latissima*) AND WINGED KELP (*Alaria esculenta)*

3.1. Justification and Objectives

Efforts are in underway in the New England area to develop a sustainable sea vegetable industry. The growing demand for local, farm-raised and fresh healthful foods (Sloan 2015) shows great potential for a fresh sea vegetables market. Several species of brown sea vegetables, including sugar kelp and winged kelp, are currently being cultivated in the Gulf of Maine. The recent increase in their consumption has prompted sea vegetable farmers to create and sell diverse fresh sea vegetable products. However, factors causing or contributing to quality loss of fresh farm-raised brown sea vegetables have been inadequately examined. Additionally, prior work on nutritional content of brown sea vegetables has focused on the commercially dried and wild harvested forms. As sea vegetable producers begin to move toward selling aquacultured fresh brown sea vegetables, more reports are needed on their nutritional content.

The overall aims of this study were to assess shelf life of refrigerated farm-raised sugar kelp and winged kelp, and to assess their nutritional content on freshly harvested samples. Two refrigerated temperatures, 35 ºF and 45 ºF, were used in in study to store the sea vegetables. The lower temperature, 35 ºF, is recommended for most fresh vegetables whereas the higher temperature, 45 ºF, is closer to the conditions observed in restaurant refrigerators, which are opened and closed multiple times throughout the day. Since sea vegetable producers are creating different product forms with various sea

vegetables, it was also key to examine whether shredding to produce a "salad cut" would affect the shelf life of these species. Additionally, there have been no previous studies reported on the effects of harvest season on storage of fresh sea vegetables. The specific objective of this study was to assess quality deterioration of fresh farm-raised sugar kelp (February and June harvest) and winged kelp (whole fronds and shredded slaw) at 35 ºF and 45 ºF for up to two weeks or until samples were unfit for human consumption by 1) sensory evaluation, 2) microbiological assays, 3) physicochemical quality (color, texture, drip loss, soluble protein content and total volatile base nitrogen content). A second objective was to determine the major chemical constituents and vitamin C content of fresh, farm-raised sugar kelp and winged kelp.

3.2. Materials and Methods

3.2.1. Shelf Life Studies

Three separate shelf life studies were conducted on sugar kelp*, Saccharina latissima* (February (SK) and June harvest (SK2)) and winged kelp (Al), *Alaria esculenta* (April harvest), based on their availability. Effects of (a) two refrigeration temperatures, 35 ºF and 45 ºF, (b) product form, whole fronds (WF) and shredded slaw (SS), and (c) harvest season (Table 3.1), on sensory, microbial, and physicochemical properties of samples were assessed every 2-3 days for up to 2 weeks or until samples were unfit for human consumption. Freshly harvested crops from a Clark Cove farm leased by Maine Fresh Sea Farms (Bristol, Maine), were cleaned with sea water and packaged into 2-gallon ziploc bags and delivered in coolers on ice the next day for sugar kelp (February) and the same day for sugar kelp (June) and winged kelp.

Table 3.1. Experimental treatments and codes.

Species	Temperature	Product form	Harvest season
Sugar kelp	35 ºF 45 ºF	WF (whole fronds) SS (shredded slaw)	**SK** (Feb) **SK2** (June)
Winged kelp (Al)	35 ºF 45 ºF	WF (whole fronds) SS (shredded slaw)	N/A

N/A= not applicable.

For sugar kelp (February), all analyses except instrumental texture were performed on days 1,3,5,7,9 and 11 of storage. There was no sensory evaluation on day 5 since it was during the weekend and panel members were unavailable. Instrumental texture was assessed on days 4,6 and 8. For sugar kelp (June) and winged kelp, all analyses except instrumental texture were performed on days 1,3,5,8,10 and 12. Instrumental texture was assessed on days 2,4,6,9,11 and 13. All the processing was performed in triplicate (A, B, C).

3.2.1.1. Sample Preparation

Approximately 15 kg of sugar kelp harvested in February, 18 kg of sugar kelp harvested in June and 21 kg of winged kelp were delivered in coolers. Fresh samples were divided (Table 3.2) into twelve 2.5-gallon plastic ziploc bags. There were 2 bags per replicate, with a total of 24 bags for each species. Any sea vegetable that were degraded or decayed were removed from the study prior to packaging. For shredded slaw, samples were manually cut with a chef's knife. Once cut, they were weighed into the Ziploc bags. The bags were stored in coolers on ice until all the bags were prepared and then the

samples were stored in two separate refrigerators; one held at 35 ºF and another at 45 ºF. The bags were randomly placed on the refrigerator racks to avoid any effects due to potential uneven refrigerator temperatures. This day was considered as Day 0 of the shelf life study. For each testing day, the required amount of sample was taken out from each replicate bag in the morning and was stored in coolers on ice while analyses were in process. Plastic trays were used to separate samples from the ice in the cooler to avoid chilling injury. Refrigerator temperature was recorded each testing day and was adjusted as needed to maintain appropriate storage conditions (35 ºF or 45 ºF).

Table 3.2. Amount of sample per bag

Sample	**Amount per bag (g)**
SK WF	585
SK SS	525
SK2 WF	590
SK2 SS	680
Al WF	545
Al SS	450

3.2.1.2. Sensory Evaluation

Twelve subjects, 18 years of age or older, who were familiar with seaweed products were recruited from the University of Maine community via word of mouth. Participants interested in sea vegetables and committed to attending a majority of the test days during each 2-week shelf life experiment were included and briefly trained on each species to evaluate specific quality attributes. During the shelf life studies, samples were rated based on visual observation and touch. The evaluation sheet (Appendix D, E, F and G) comprised a 15 cm unstructured line scale for each attribute of interest, where 0 was

the poor quality score and 15 was the excellent quality score. Opposite descriptors were attached to either ends of the line scale. The descriptors were developed based on preliminary assessment and discussion with the sensory panel. The panelists were provided with an informed consent form (Appendix C) prior to participation. Approval for research with human subjects was obtained from the Institutional Review Board (IRB) prior to conducting sensory analyses. Each day set amount of sample for each treatment (10 g for whole fronds and 15 g for shredded slaw) were taken out from each replicate bag and pooled together on a white ceramic plate for whole fronds and ceramic bowl for shredded slaw. Each plate was labeled with a 3-digit randomized code for each day. The testing took place under normal white light. Panelists were encouraged to write comments in the comments section of the evaluation sheet. The recorded ratings were measured using a 15-cm ruler.

3.2.1.3. Aerobic Plate Counts (APC)

Each testing day approximately 16 g of samples were taken out from each treatment replicate to determine aerobic plate counts (APC). Aseptic techniques were employed to place 15 g of sample in a stomacher bag with filter and 0.1% autoclaved bactopeptone (BD Diagnostics, Sparks, MD) (1:10 w/v). The samples were mechanically mixed for 2 min using a BAGMixer 400 (Model P, SpiralBiotech, Advanced Instruments, Norwood, MA). Initially, one mL aliquots of three serial dilutions (1:10, 1:100 and 1:1000) were plated on Petrifilm™ Aerobic Count Plates (3M, St. Paul, MN). The plates were incubated for 48 h at 37 ºC after which only films with 30-300 colonies were recorded. The dilutions were increased as necessary depending on the data. All the three replications for each treatment were analyzed in duplicate and the values were averaged.

To obtain the colony forming units (CFU) of bacteria per gram of sample, the plate count number was multiplied with the dilution factor.

3.2.1.4. Physicochemical Analyses

3.2.1.4.1. Colorimetric Analyses

Colorimetric analyses were performed using a Hunter L*a*b* colorimeter (LabScan XE, Hunter Labs, Reston, VA) in which L* value is based on a scale of dark (0) to light (100), a* value is based on a scale of green (-) to red (+), and b* value is based on a scale of blue (-) to yellow (+). Black and white ceramic standard plates were used to standardize the colorimeter before each use and the colorimeter was allowed to warm up for 30 min prior to color analysis. A port size of 50.5 mm, area view of 44.5 mm, and D65 illumination were used. The disc with a 5.1 cm diameter hole was used. Each testing day ~90 g of sample was used for obtaining 10 readings in total from each replicate. One layer of sample was spread on the 2.5-inch diameter colorimeter glass cup to cover the base of the cup. Each sample was read 3 times by rotating the colorimeter cup 120º after the initial reading, and the values were averaged to provide one reading per sea vegetable sample.

3.2.1.4.2. Texture Analyses

Both the species under consideration were different from each other anatomically. The tests developed were species and product form specific.

3.2.1.4.2.1. Texture Profile Analysis (TPA)

Eighty grams of sugar kelp, whole fronds (WF), samples were cut in to 3 cm x 3 cm squares using a cookie cutter, and then stacked to 0.8 cm in height. The texture analyzer (TA-XTi2, Texture Technologies Inc., Scarsdale, NY) was calibrated using a

5,000 g load cell from the same company. A 2-inch diameter cylindrical probe was used with 1 mm/sec pre-test speed, 2 mm/sec test-speed and post-test speed and with a distance of 3 mm. Force in Newtons (N) was recorded by the texture analysis software (Exponent 32, version 5.0, 6.0, 2010, Texture Technologies Inc., Scarsdale, NY). A total of 8 values for SK and 10 values for SK2 and Al were averaged per treatment replicate.

3.2.1.4.2.2. Knife Blade Shear Force

A shear test using a 6 cm Craft knife blade was used to measure the force (N) to cut the winged kelp whole fronds. The texture analyzer (TA-XTi2, Texture Technologies Inc., Scarsdale, NY) was calibrated using a 5,000 g load cell from the same company before each use. Pre-test speed was 1mm/sec, test and post-test speed was 2 mm/sec and distance was 4 mm. The knife blade cut through a single 3 cm x 3 cm square of winged kelp that was cut from the middle of the winged kelp to incorporate the midrib as well as the blade. Texture analysis software (Exponent 32, version 5.0, 6.0, 2010, Texture Technologies Inc., Scarsdale, NY) was used to take 10 values per treatment replicate, which were averaged.

3.2.1.4.2.3. Compression Test

A compression test using a 1/2-inch diameter cylindrical probe was used to measure force (N) for SK, SK2 and Al shredded slaw samples. The texture analyzer (TA-XTi2, Texture Technologies Inc., Scarsdale, NY) was calibrated using a 5,000 g load cell from the same company before each use. Pre-test speed was 1mm/sec, test and post-test speed was 2 mm/sec and distance was 6 mm. Samples were filled to the top of a round plastic cup with a diameter of 50 mm and height of 7 mm. Texture analysis software

(Exponent 32, version 5.0, 6.0, 2010, Texture Technologies Inc., Scarsdale, NY) was used to take 10 values per treatment replicate, which were averaged.

3.2.1.4.3. Drip Loss

During the shelf life study of sugar kelp harvested in February, drip loss was observed over time. However, at that time it was not a dependent variable in the study. Drip loss was added as a variable for further studies to assess how much tissue fluids were lost during storage. Triplicate batches of 100 g of winged kelp and 250 g of sugar kelp (June) per treatment were stored in separate 1-gallon ziploc bags. Drip loss was measured by removing and weighing all the tissue fluids. The bag was tilted for 30 seconds to remove the cellular liquid. Percent water loss was calculated from the measurements.

$$\% \text{ drip loss} = \frac{\text{fluid loss (g)}}{\text{initial sample weight (g)}} \times 100$$

3.2.1.4.4. Soluble Protein

Soluble protein was extracted using the methods described by Paull and Chen (2008) with slight modifications. Eight grams of sample was chopped and homogenized with 32 mL phosphate buffer (pH 7) using a Polytron homogenizer (Brinkmann Instruments, Westbury, NY) for two minutes. Homogenized samples were subjected to centrifugation (Beckman J-25, Brea, CA) at 14000 xg for 15 minutes. The supernatant was frozen at -20 ºC until further analyses. Protein analysis was performed as described by Lowry and others (1951). Briefly, 5 mL of freshly prepared solution with 2% Na_2CO_3 (Sigma Aldrich, St. Lois, MO) in 0.4% NaOH, 1% cupric sulfate (Fisher Scientific, Waltham, MA) and 2.7% sodium potassium tartrate (Fisher Scientific, Waltham, MA) in

ratio 100:1:1 was added to 100 µL of sample extract and incubated for 10 min. Another 500 µL of Folin's Ciacalteu Reagent, 2N (Fisher Scientific, Waltham, MA) diluted with distilled water 1:2 was added and incubated for 40 min. A series of bovine serum albumin standards were used for calculation of soluble protein content. Distilled water (100 µL) was used as the blank. Absorbance was read at wavelength 700 nm using a spectrophotometer (Beckman Du 530, Brea, CA).

3.2.1.4.5. Total Volatile Base Nitrogen (TVBN)

TVBN is a common method of assessing microbial spoilage in seafood such as fish and shellfish. Microorganisms present in seafood produce volatile amines including trimethylamines, dimethylamines and ammonia which increase with microbial spoilage. Fifteen grams of samples were homogenized with 30 mL of 7.5% trichloroacetic acid (TCA) (Fisher Scientific, Waltham, MA) for 1 min in a Magic Bullet (Nutribullet, USA). The mixture was centrifuged (Eppendorf Model 5430, Hamburg, Germany) at 1878 xg for 20 minutes and supernatant was frozen until further analysis. The supernatant was thawed before analysis and 15 mL of it was added to the micro-Kjeldahl distillation unit (Rapid distillation unit, Labconco, Kansas City, MO). An indicator was prepared by mixing 0.2% methyl red and 0.2% methylene blue (2:1) in ethanol. A blank was prepared with 20 mL TCA and 6 mL distilled water. Four mL of 10% sodium hydroxide (EM Sciences, USA) were slowly added to the receiving flask. Samples were distilled into 15 mL of 4% boric acid (Fisher Scientific, Waltham, MA) containing 8 drops of indicator to a final volume of approximately 40 mL. The distillate was then titrated using 0.05 N hydrochloric acid until a constant purple color was obtained (Fisher Scientific, Waltham, MA). An internal standard of ammonium sulfate and trimethylamine-HCl containing

4.26% nitrogen/mL was run to ensure that the method was running accurately. The amount of TVBN (mg/100g of wet sample) was calculated as follows:

[(volume (mL) HCl required for titrating sample – volume (mL) HCl used for titrating blank) x HCl normality) x molecular weight of N] x [(volume of extraction solution/ volume of extract used for distillation) x (100/ original weight (g) of sample)]

3.2.1.5. Statistical Analyses

Data were analyzed using JMP 12.2 (SAS Software, Cary, NC). Shapiro-Wilk's normality test and Levene equality of variances were used to assess data prior to further analyses. Multiway ANOVA was used to assess overall effects of time and treatment. One-way analysis of variance (ANOVA) was selected to find treatment differences each day. Tukey's Honest Significant Difference (HSD) test was selected for post-hoc analyses. In cases where data did not satisfy normality, homogeneity or independence, they were transformed logarithmically. In cases where transformation failed to satisfy data distribution assumptions, data were analyzed non-parametrically using Kruskall-Wallis. Steel-Dwass test was selected for post-hoc analyses post non-parametric analyses. A significance level of $p<0.05$ was chosen for all statistical analyses.

3.2.2. Nutritional Analyses

3.2.2.1. Sample Preparation

Approximately 500 g of fresh sea vegetables were pureed using a food processor and dried in a convection oven (VWR International, Radnor, PA) at 105 ºC until reaching a constant weight. The dried sample was crushed and ground further by using a motor and pestle. The ground sample was stored in a whirlpack bag in a desiccator until nutritional analysis. Three subsamples for each analysis were used from this homogenous

powder. Moisture content and Vitamin C content were analyzed on freshly pureed samples.

3.2.2.2. Moisture Content

Moisture content of pureed sea vegetables was determined gravimetrically according to the AOAC method 950.46 by drying 5 g sample in a pre-weighed aluminum pan in triplicate overnight in a convection oven at 105 ºC (VWR International, Radnor, PA) (AOAC 2005). Pans containing the samples were re-weighed and the percent moisture was calculated as follows:

$$\text{g/100g Moisture} = \frac{[\text{pan wt. (g)} + \text{wet sample wt. (g)}] - [\text{pan wt. (g)} + \text{dry sample wt. (g)}]}{\text{wet sample wt. (g)}} \times 100$$

3.2.2.3. Ash Content (Total Minerals)

Ash content was also determined gravimetrically according to the AOAC method 938.08 (AOAC 2005). Two hundred mg of oven-dried sample in a pre-weighed scintillation vial was charred on a hot plate set on medium. The samples were charred until there was no smoke coming out of the samples. The sample vials were then placed in a muffle oven (Thermolyne Model F-A1730, Dubuque, IA) at 550 ºC for six hours. Vials containing the samples were re-weighed and the percent ash on dry basis (dwb) was calculated as follows:

$$\text{g/100g Ash} = \frac{[\text{vial wt. (g)} + \text{ash wt. (g)}] - \text{vial wt. (g)}}{\text{dry sample wt. (g)}} \times 100$$

3.2.2.3.1. Selected Minerals

The ashed samples in scintillation vials were dissolved with 7 mL of concentrated *omni*trace nitric acid (EM Science, USA) and 1 mL of hydrochloric acid (Fisher

Scientific, Waltham, MA). After the bubbling of samples had stopped, approximately 10 mL of distilled water was added and the samples were vortexed for approximately 5 s. The contents of the vial were poured into a 100 mL quantitative flask and brought to volume with distilled water, stirred, and allowed to settle overnight. Approximately 10-15 mL of each sample was poured into a new pre-labelled scintillation vial and then analysed by inductively coupled plasma atomic emission spectroscopy (ICP-OES) (Thermo Elemental IRIS Interpid DUO ICP-OES, USA) to determine calcium, potassium, magnesium phosphorous, aluminum, copper, iron, sodium and zinc. All the samples were analyzed in triplicate.

3.2.2.4. Crude Fat Content

The fat content was determined by AOAC the acid hydrolysis method 948.15 (AOAC 2005). Two and a half grams of oven-dried samples were added in French Square bottles with 10 mL of 8.1 N hydrochloric acid (Fisher Scientific, Waltham, MA) and were placed in a water bath at 85-90 ºC for 90 minutes. The samples were cooled prior to adding 7 mL of ethanol (Fisher Scientific, Waltham, MA) and swirling for 15 s. Twenty-five mL of ethyl ether (Fisher Scientific, Waltham, MA) were added to the sample and shaken for 60 s. For the first 15 s the samples were moderately shaken and then vigorous shaking followed for 45 s. Following this, twenty-five mL of petroleum ether (Fisher Scientific, Waltham, MA) were added to the sample and shaken for 60 s in the same fashion. The samples were then allowed to settle for at least 30 min to allow the emulsion to break. The top layer (ether plus fat) was carefully extracted using a glass pipette and transferred to a pre-weighed flat bottom beaker. Three more extractions were performed using 15 mL of ethyl ether and petroleum ether followed by shaking and

adding the top layer to the previously collected pool. The pooled ether with lipid was allowed to dry overnight under the chemical hood followed by drying in a 105 ºC the oven for 7. The fat content (dwb) was calculated by reweighing the cooled beakers and using the following formula:

$$\text{g/100g Crude Fat} = \frac{[(\text{flask (g)} + \text{fat weight (g)}) - \text{flask weight (g)}]}{\text{sample weight (g)}} \times 100$$

3.2.2.5. Crude Protein Content

The nitrogen content of the dried samples was determined by combustion analyzer (TRU MAC CNS, LECO Corp., MI, USA) using oven-dried samples. The crude protein content (dwb) was calculated by multiplying the nitrogen content by a conversion factor of 6.25 used for seafood. Each analysis was performed in duplicate.

3.2.2.6. Carbohydrate Content

The carbohydrate content of the samples was calculated by difference as follows:

g/100g Carbohydrate = 100 – (ash content + fat content + protein content)

3.2.2.7. Vitamin C

Vitamin C was determined by following AOAC method 967.21 and 985.33, titrating sample extracts using 2,6-dichlorophenolindophenol dye (AOAC 2005). Eight grams of fresh pureed sample were homogenized with 15 mL precipitant solution using a Polytron homogenizer (Brinkman Instruments, Westbury, NY) for 2 min and centrifuged (Beckman J-25, Brea, CA) at 10,000 xg for 15 min at 4 ºC. The pellet was re-suspended in 15 mL precipitant solution and centrifuged again. The supernatants were pooled together and the final volume was recorded. The precipitant solution was made by mixing equal amounts of two solutions. The first solution was made by dissolving 15 g of glacial meta-phosphoric acid in 40 mL glacial acetic acid and bringing it to 250 mL with

distilled water. The solution was filtered using a P8 qualitative paper (Fisher Scientific, Waltham, MA). The second solution was made by dissolving 0.9 g ethylene diamine tetra acetic acid (EDTA) (Sigma Aldrich, St. Lois, MO) in 200 mL of distilled water and bringing it up to 250 mL. The precipitant solution was made fresh on the day of use. Ascorbic acid (1 mg/mL) was used the standard solution and was prepared fresh by diluting 50 mg ascorbic acid (Fisher Scientific, Waltham, MA) to 50 mL with the precipitant solution in a volumetric flask. For the indophenol solution, 0.0625 g of 2,6-dichlorophenolindophenol sodium salt and 0.0525 g of sodium bicarbonate (Fisher Scientific, Waltham, MA) were brought up to 250 mL with distilled water. After mixing thoroughly, the solution was passed through a fisher P8 filter (Fisher Scientific, Waltham, MA). The ascorbic acid standard plus 5 mL precipitant solution was titrated using the indophenol dye until rose pink color persisted for 10 s. Fifteen mL aliquots of sample extracts were poured in 50 mL Erlenmeyer flasks and titrated with the indophenol dye until the rose-pink endpoint lasted for 10 s. For the sample blank, two 15 mL aliquots of precipitant solution were added into separate 50 mL Erlenmeyer flasks and titrated with indophenol standard solution to obtain the same endpoint. The of ascorbic acid concentration of the sample was calculated using the following formula:

mg of ascorbic acid/g or mL of sample = C x V x (DF/WT)

where, C = mg of ascorbic acid/mL of dye,

V = mL of dye used for titration of diluted sample (subtract blank volume first),

DF = dilution factor and

WT = sample weight (g)

3.2.2.8. Data Presentation

Analytical replicates were averaged (± standard error) and reported on dry weight basis, except for moisture and vitamin C content. The macronutrients were presented in g/100g whereas the micronutrients and vitamin C were presented in mg/100g.

3.3. Results and Discussion

The three shelf life studies on sugar kelp and winged kelp provided new and useful information on their quality changes during refrigerated storage. For both the species, it was observed that the smaller fronds deteriorated much faster than the bigger, more mature fronds. The blades of small fronds wilted faster compared to that of bigger winged kelp fronds. In particular, the initial crisp midrib texture of smaller winged kelp fronds deteriorated faster than in the bigger fronds. Freshly harvested sugar kelp harvested in June had a slimier surface than the February harvest when received. Additionally, the summer harvest whole fronds were noticeably bigger and thicker than the winter harvested sugar kelp fronds. It is important to note that during the unusually cold 2014-2015 winter in Maine water temperature dropped below the freezing point several times during the growing season. This could have resulted in freezing of the February sugar kelp crop, forming icicles which causes cellular damage and contributed to the rapid quality deterioration postharvest. The sea vegetables had a fresh, ocean aroma when they were first received but the aroma was more intense for winged kelp compared to sugar kelp. Considerable drip losses, upon receiving the samples, were observed during all three studies but sugar kelp harvested in February had the greatest loss.

3.3.1. Shelf life studies

3.3.1.1. Sensory Evaluation

A 15 cm unstructured scale with opposite descriptors at either end was used to rate sensory color, aroma, texture and overall quality (Appendix D, E, F, G). The descriptors were determined during the training sessions based on the panelists' suggestions and agreement. The best quality score was 15 and the lowest quality score was 0. For both the kelps, sensory color descriptors went from dark brown-green (score 15) to faded brown-green (score 0) whereas for aroma, the descriptors used were pleasant (15) and unpleasant (0) for both the species. For sugar kelp texture, the scale ranged from strong (15) to fragile (0). For texture of winged kelp fronds, both the blade and midrib texture were rated. The blade texture descriptors ranged from strong (15) to fragile (0) whereas the midrib texture ranged from crisp (15) to limp (0). For winged kelp shredded slaw, the texture descriptors used were firm (15) versus mushy (0). Sheen was assessed as an attribute using descriptors dull (0) and glossy (15) only for sugar kelp. Overall quality for both species and product forms was assessed with fresh (15) and complete loss of freshness (0) as descriptors.

The sensory color scores for sugar kelp harvested in February (SK) were significantly ($p<0.01$) affected only by time, with scores decreasing towards the end of the study. For all the SK treatments, the values dropped significantly by day 7 (Table 3.3). For sugar kelp harvested in June (SK2), both time and higher temperature significantly decreased the sensory color scores. Sensory color scores were similar for shredded slaw samples stored at 35 ºF and 45 ºF, indicating that temperature did not affect the sensory color scores for the slaw although it did for the whole fronds. Both

time and higher storage temperature significantly decreased sensory color values for winged kelp (Al). Winged kelp samples stored at 45 ºF received lower scores as time progressed in comparison to samples stored at 35 ºF.

Table 3.3. Sensory color scores for sugar kelp February harvest (SK), sugar kelp June harvest (SK2) and winged kelp (Al) during refrigerated storage.

Day	**SK WF 35**	**SK WF 45**	**SK SS 35**	**SK SS 45**
1	11.98 ± 0.42b	12.15 ± 0.54b	12.74 ± 0.47b	12.56 ± 0.35b
3	10.46 ± 0.83b	9.80 ± 0.76ab	12.37 ± 0.39b	12.53 ± 0.50b
7	5.59 ± 1.08a	5.34 ± 1.33a	8.19 ± 1.41a	7.18 ± 1.36a
Day	**SK2 WF 35**	**SK2 WF 45**	**SK2 SS 35**	**SK2 SS 45**
1	11.93 ± 0.68ab	12.06 ± 0.70c	12.94 ± 0.66b	12.29 ± 0.92b
3	12.49 ± 0.68b	12.38 ± 0.60bc	12.88 ± 0.47b	12.25 ± 0.51b
5	9.29 ± 1.36ab	8.24 ± 1.20ab	10.64 ± 0.93ab	9.16 ± 1.14ab
8	9.80 ± 0.94ab	9.43 ± 0.68bc	8.61 ± 1.01ab	9.09 ± 0.88ab
10	10.03 ± 0.91ab	6.09 ± 1.14a	8.84 ± 1.29ab	6.89 ± 1.06a
12	7.95 ± 1.36a	5.35 ± 1.06a	7.31 ± 1.31a	7.25 ± 1.39a
Day	**Al WF 35**	**Al WF 45**	**Al SS 35**	**Al SS 45**
1	11.06 ± 0.68c	11.91 ± 0.62b	12.84 ± 0.36c	12.09 ± 0.49b
3	11.26 ± 0.59c	10.67 ± 0.71b	11.08 ± 0.79bc	10.72 ± 0.69b
5	9.46 ± 0.63bc	8.93 ± 1.23b	10.30 ± 0.78bc	9.01 ± 1.53ab
8	10.01 ± 0.31c	5.56 ± 0.92a	9.79 ± 1.22abc	8.39 ± 1.16ab
10	7.29 ± 0.66b	n.d	8.54 ± 0.81ab	6.86 ± 1.06b
12	5.22 ± 0.74a	n.d	6.72 ± 1.03a	5.12 ± 0.96b

Each value is the mean ± standard error, (n=8-12, depending on test day). Values not sharing a letter are significantly ($p<0.05$) different within columns, within each treatment, analyzed by ANOVA followed by Tukey's HSD post hoc test or analyzed non-parametrically by Kruskall-Wallis followed by Steel-Dwass post hoc test. n.d=not determined

Duration of storage had a significant effect on sensory aroma scores for sugar kelp harvested in February, with values dropping significantly by day 7 for all the treatments (Table 3.4). Both time and temperature significantly affected sugar kelp

harvested in June but no significant effects of product form were observed. Sensory aroma scores for SK2 whole fronds stored at 35 ºF did not change significantly over time, however, they dropped significantly for samples stored at 45 ºF by day 10. Moreover, scores for SK2 shredded slaw dropped faster at 45 ºF compared to 35ºF. These results clearly indicate that 35 ºF storage delayed the onset of a more unpleasant aroma. Significant effects of time and temperature were also observed for sensory aroma scores of winged kelp, with decreasing scores over time and with higher temperature. Panelists mentioned that they detected a “sour odor” or “unpleasant odor” as the sensory aroma scores dropped.

Table 3.4. Sensory aroma scores for sugar kelp February harvest (SK), sugar kelp June harvest (SK2) and winged kelp (Al) during refrigerated storage.

Day	SK WF 35	SK WF 45	SK SS 35	SK SS 45
1	12.23 ± 0.93b	11.56 ± 1.05b	12.66 ± 0.68b	12.49 ± 0.76b
3	11.07 ± 1.27ab	11.17 ± 1.00b	12.28 ± 1.90b	12.18 ± 1.19b
7	8.07 ± 1.10a	4.54 ± 1.38a	9.49 ± 1.62a	5.42 ± 1.26a
Day	**SK2 WF 35**	**SK2 WF 45**	**SK2 SS 35**	**SK2 SS 45**
1	13.06 ± 0.46a	13.40 ± 0.30c	13.80 ± 0.17b	13.48 ± 0.40c
3	12.81 ± 0.46a	12.53 ± 0.59c	12.51 ± 0.58b	12.66 ± 0.51bc
5	11.81 ± 0.93a	11.09 ± 0.93bc	11.04 ± 0.87ab	11.66 ± 0.88bc
8	11.34 ± .076a	9.94 ± 0.92abc	10.30 ± 0.92ab	9.48 ± 1.25b
10	9.53 ± 0.83a	8.53 ± 1.33ab	10.32 ± 1.41ab	9.46 ± 1.30b
12	9.09 ± 1.43a	7.16 ± 1.00a	8.74 ± 1.39a	5.04 ± 1.02a
Day	**Al WF 35**	**Al WF 45**	**Al SS 35**	**Al SS 45**
1	10.86 ± 0.88b	12.28 ± 0.66c	11.96 ± 0.74c	11.61 ± 0.74c
3	11.16 ± 0.72b	11.24 ± 0.80bc	10.32 ± 0.82bc	11.46 ± 0.75c
5	9.74 ± 0.92b	8.18 ± 1.23ab	9.99 ± 0.66bc	7.19 ± 1.16bc
8	9.13 ± 0.75b	6.53 ± 0.97a	7.51 ± 1.32ab	9.01 ±1.34ab
10	8.55 ± 0.93b	n.d	7.45 ± 0.97ab	5.44 ± 1.07ab
12	5.10 ± 0.87a	n.d	4.49 ± 0.65a	4.20 ± 0.64a

Each value is the mean ± standard error, (n=8-12, depending on test day). Values not sharing a letter are significantly ($p<0.05$) different within columns, within each treatment, analyzed by ANOVA followed by Tukey's HSD post hoc test or analyzed non-parametrically by Kruskall-Wallis followed by Steel-Dwass post hoc test. n.d=not determined

Sensory texture scores were significantly affected by time, temperature and product form for sugar kelp harvested in February. The texture scores dropped drastically by day 7, especially at 45 ºF (Table 3.5). Shredded slaw scored slightly better compared to whole fronds over time. The panelists commented that by day 7 the texture had degraded tremendously, leaving a "mushy gunk." However, sugar kelp harvested in June maintained its texture much better over time compared to the winter harvest. Also although its sensory texture scores dropped significantly over time, temperature and

product form did not affect the scores. Seasonal effects were prominent, indicating that sugar kelp harvested in June kept its original texture better compared to the February crop. Time and temperature affected blade texture scores for winged kelp samples, with lower scores for samples stored at 45 ºF. The blade, attached to the midrib, deteriorated so much that by day 10 only the midrib was left and hence that treatment was taken out of the study. Time significantly affected texture scores of shredded slaw winged kelp samples, however, temperature had no effect. Towards the end of the study, shredded slaw samples received lower scores compared to the beginning, moving towards the mushy end of the 15 cm line scale.

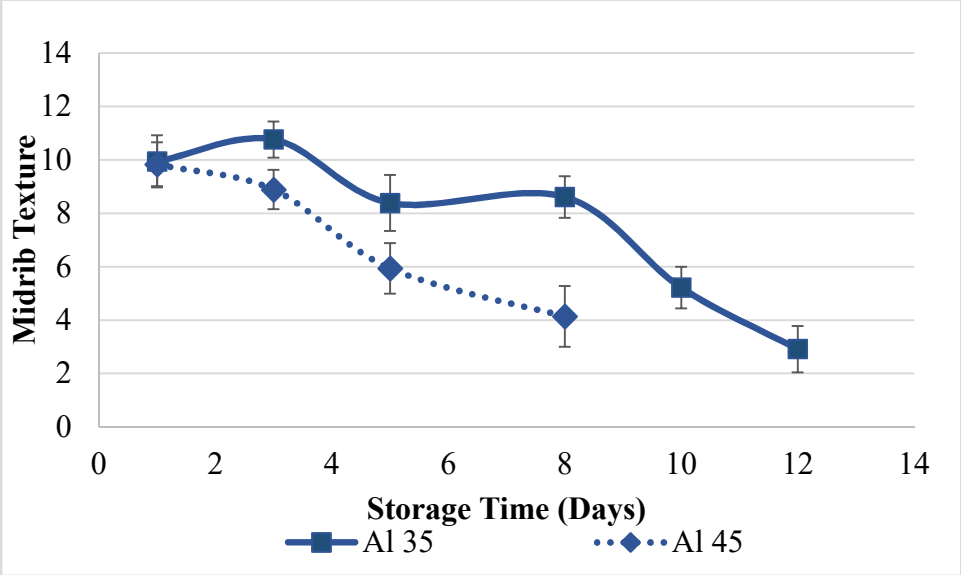
Table 3.5. Sensory texture scores for sugar kelp February harvest (SK), sugar kelp June harvest (SK2) and winged kelp (Al) during refrigerated storage.

Day	SK WF 35	SK WF 45	SK SS 35	SK SS 45
1	11.74 ± 1.04b	12.66 ± 0.51b	11.89 ± 0.76b	12.33 ± 0.93b
3	12.59 ± 0.32b	11.50 ± 0.45ab	12.43 ± 0.48b	12.21 ± 0.36b
7	4.31 ± 1.29a	1.55 ± 0.45a	8.83 ± 1.52a	3.36 ± 0.85a
Day	**SK2 WF 35**	**SK2 WF 45**	**SK2 SS 35**	**SK2 SS 45**
1	13.46 ± 0.27b	13.50 ± 0.46b	13.56 ± 0.19c	13.43 ± 0.45c
3	12.25 ± 0.70b	12.75 ± 0.49b	12.74 ± 0.54c	12.79 ± 0.38c
5	11.60 ± 0.79b	10.81 ± 0.83ab	10.31 ± 0.67abc	11.17 ± 0.55bc
8	10.74 ± 0.77ab	9.99 ± 0.91ab	10.94 ± 0.58bc	8.34 ± 0.71ab
10	10.50 ± 0.74ab	7.99 ± 1.19a	8.81 ± 1.36ab	7.94 ± 1.32ab
12	7.41 ± 1.58a	8.11 ± 1.46a	6.81 ± 1.50a	7.61 ± 1.33a
Day	**Al WF 35**	**Al WF 45**	**Al SS 35**	**Al SS 45**
1	11.81 ± 0.55c	12.31 ± 0.55c	12.42 ± 0.49d	10.90 ± 1.02c
3	11.41 ± 0.57c	8.76 ± 0.94b	9.58 ± 0.78bc	11.21 ± 0.74c
5	7.34 ± 0.91b	5.21 ± 0.82a	10.20 ± 0.66cd	6.61 ± 1.12ab
8	7.5 ± 0.68b	4.17 ± 1.01a	6.89 ± 0.83ab	8.44 ± 1.06bc
10	6.59 ± 0.76b	n.d	6.35 ± 0.49a	5.46 ± 0.78ab
12	2.77 ± 0.83a	n.d	4.60 ± 0.69a	3.96 ± 0.71a

Each value is the mean ± standard error, (n=8-12, depending on test day). Values not sharing a letter are significantly ($p<0.05$) different within columns, within each treatment, analyzed by ANOVA followed by Tukey's HSD post hoc test or analyzed non-parametrically by Kruskall-Wallis followed by Steel-Dwass post hoc test. n.d=not determined

Since winged kelp had a thick midrib to which the blades were attached, quality of midrib texture was also rated during storage. Panelists were asked to snap the midrib and rate its crispness. Sensory scores for this attribute were significantly affected by time and temperature, where the scores decreased over time but at a faster rate for samples stored at 45 ºF (Fig 3.1). These results were similar to the blade texture scores, indicating that the overall texture was maintained better at the lower storage temperature. According to the sensory panel the midrib became "limp" and "bendy" over time.

Figure 3.1. Sensory midrib texture scores for winged kelp during refrigerated storage.



Each value represents the mean ± standard error, (n=8-12, depending on test day).

For sugar kelp, sheen was also assessed as a sensory attribute. The scores dropped significantly over time for both, February and June, sugar kelp harvests, indicating that the samples were becoming dull in appearance (Table 3.6). However, for both crops, no effects of temperature or product form were observed.

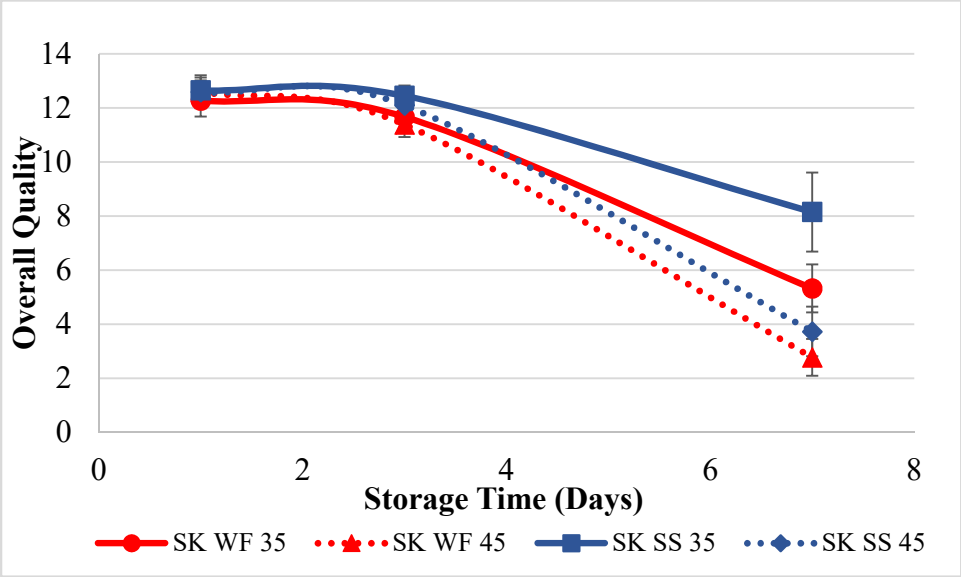
Table 3.6. Sensory sheen scores for sugar kelp February harvest (SK) and sugar kelp June harvest (SK2) during refrigerated storage.

Day	SK WF 35	SK WF 45	SK SS 35	SK SS 45
1	11.80 ± 0.86b	12.14 ± 0.62a	12.15 ± 1.13b	11.76 ± 0.62b
3	11.39 ± 0.33b	9.93 ± 1.17a	12.63 ± 0.37b	11.70 ± 0.68b
7	6.50 ± 0.83a	3.33 ± 0.64a	9.24 ± 1.41a	5.81 ± 1.23a
Day	**SK2 WF 35**	**SK2 WF 45**	**SK2 SS 35**	**SK2 SS 45**
1	13.30 ± 0.43a	12.99 ± 0.58c	13.60 ± 0.31c	13.45 ± 0.35c
3	12.64 ± 0.48a	12.38 ± 0.61c	12.73 ± 0.54c	12.77 ± 0.44bc
5	11.56 ± 0.99a	9.47 ± 1.57abc	11.87 ± 0.52bc	11.81 ± 0.71bc
8	10.77 ± 0.83a	10.29 ± 0.72bc	10.50 ± 0.74abc	10.24 ± 1.01abc
10	9.17 ± 0.87a	7.10 ± 1.03ab	8.50 ± 1.29ab	9.13 ± 1.35ab
12	8.56 ± 1.53a	6.45 ± 1.05a	8.04 ± 0.40a	7.71 ± 1.42a

Each value is the mean ± standard error, (n=8-12, depending on test day). Values not sharing a letter are significantly ($p<0.05$) different within columns, within each treatment, analyzed by ANOVA followed by Tukey's HSD post hoc test or analyzed non-parametrically by Kruskall-Wallis followed by Steel-Dwass post hoc test

For overall quality of sugar kelp harvested in February, scores significantly decreased over time. However, as was observed with sensory color scores, the sensory overall quality scores were not affected by temperature or product form. All the treatments received similar scores on day 1and 3, however, the scores plummeted by day 7, owing to the faded color, degraded texture and off-odor (Fig 3.2). The panelists mentioned that they would not consume the samples on day 7.

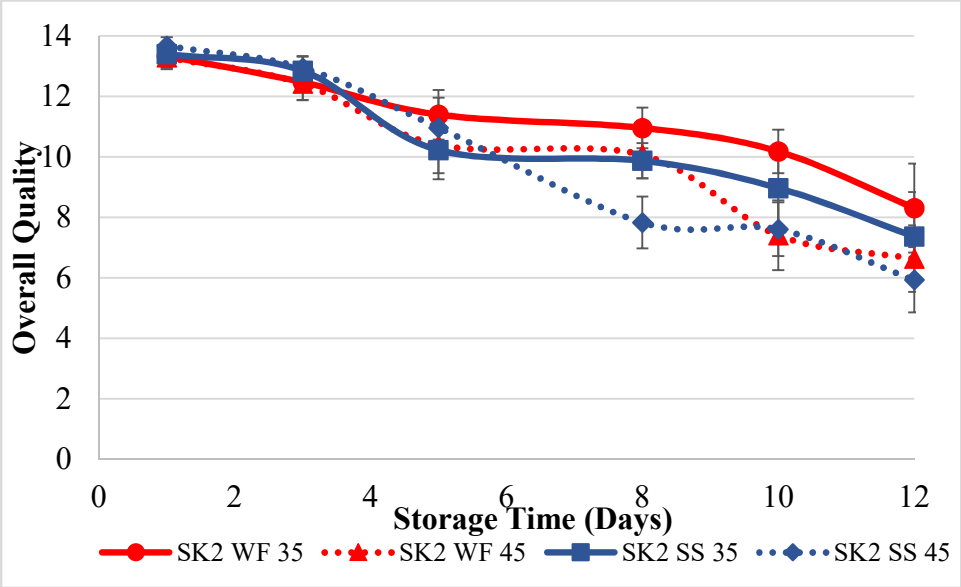
Figure 3.2. Sensory overall quality scores for sugar kelp harvested in February during refrigerated storage.



Each value represents the mean ± standard error, (n=8-12, depending on test day).

The overall quality scores for sugar kelp harvested in June were significantly affected by time and temperature but not the product form. In comparison to the winter harvest, SK2 samples received higher scores towards the end of the study, indicating that harvest season impacted overall quality. Samples stored at higher temperature were rated slightly lower towards the end of the study (Fig 3.3), however, no significant effects of time were observed for that treatment. Panelists mentioned that the whole frond samples had a “sticky/slimy” feel randomly throughout the study, coinciding with the initial observation of slimier sugar kelp fronds harvested in June.

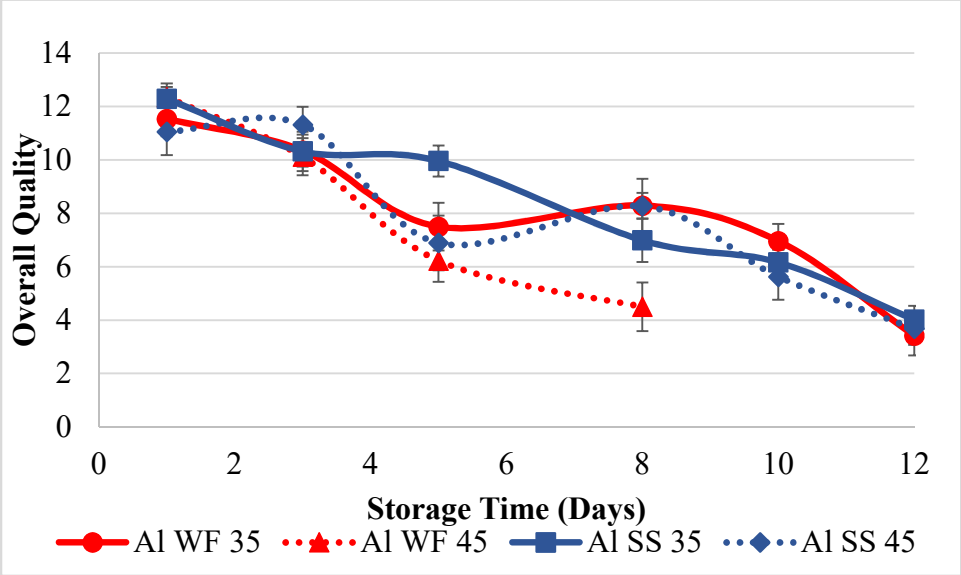
Figure 3.3. Sensory overall quality scores for sugar kelp harvested in June during refrigerated storage.



Each value represents the mean ± standard error, (n=8-12, depending on test day).

Time and temperature significantly affected overall quality scores of winged kelp samples, where the scores decreased during storage. Moreover, the lower storage temperature maintained the overall quality better than the higher storage temperature. The scores for overall quality of Al whole fronds stored at 45 ºF fell below 5 by day 8 whereas it did not fall below 5 until day 12 for whole fronds stored at 35 ºF (Fig 3.4). For shredded slaw, the scores significantly dropped by day 8 for samples stored at 35 ºF whereas at 45 ºF they dropped by day 5. These results clearly indicate that the overall quality was better at the lower storage temperature compared to the higher storage temperature.

Figure 3.4. Sensory overall quality scores for winged kelp during refrigerated storage.



Each value represents the mean ± standard error, (n=8-12, depending on test day).

3.3.1.2. Aerobic Plate Counts (APC)

Time, temperature and product form had significant effects ($p<0.01$) on the aerobic plate counts of sugar kelp harvested in February (SK) but only time affected sugar kelp harvested in June (SK2). Overall, in comparison to shredded slaw (SS), whole frond (WF) samples of SK had lower microbial activity (Table 3.7). Similar results were reported in a study comparing microbial growth of commercially available fresh-cut and whole vegetables including lettuce, spinach and endive (Abadias and others 2008). The elevated counts in the shredded slaw were likely due to chopping the whole fronds by hand, increasing the surface area, and exposing the samples to more microbes during processing. Although a significant increase in APC was observed over time for SK whole fronds, the growth was limited to 3-4 log CFU/g by the end of the study compared to 2-3 log CFU/g on day 1. These results were 3-4 log CFU/g lower than previously reported aerobic microbial counts for leafy vegetables such as iceberg and romaine lettuce (Abadias and others 2008). Slightly higher microbial counts were observed for sugar

kelp June harvest compared to the February harvest. This may be attributed to the warmer water temperatures in June compared to February, offering optimal temperatures for mesophilic bacteria. APC values increased significantly by day 10 for SK2 stored at 35ºF, whereas it increased significantly by day 8 for SK2 stored at 45ºF, possibly indicating that the higher temperature aided microbial growth in the sugar kelp. There were no significant differences over time for SK2 shredded slaw samples stored at 35ºF, however, microbial counts for samples stored at 45 ºF increased significantly by day 3, although values never exceeded 6 log CFU/g. Time, temperature and product form did not affect the microbial activity in winged kelp. Additionally, there were no significant differences in APC over time for any treatment.

Overall, these results indicate that APC were variable among species and between treatments over time. The values did not increase consistently or drastically as would be expected in refrigerator-stored leafy veggies, indicating that microbial spoilage played a secondary role in quality loss for the species studied in this study. Similar trends in microbial growth were found in the previous chapter on fresh red seaweeds and were also reported by Paull and Chen (2008) for *Gracilaria.*

Table 3.7. Aerobic plate counts (log CFU/g) for sugar kelp February harvest (SK), sugar kelp June harvest (SK2) and winged kelp (Al) during refrigerated storage.

Day	SK WF 35	SK WF 45	SK SS 35	SK SS 45
1	2.99 ± 0.17a	2.46 ± 0.17a	3.53 ± 0.27a	4.19 ± 0.12a
3	3.20 ± 0.20ab	2.83 ± 0.10a	3.48 ± 0.09a	4.52 ± 0.19a
5	3.55 ± 0.19ab	3.48 ± 0.09b	3.42 ± 0.06a	5.41 ± 0.27a
7	3.77 ± 0.12b	4.52 ± 0.19b	3.93 ± 0.12a	4.21 ± 0.24a
Day	**SK2 WF 35**	**SK2 WF 45**	**SK2 SS 35**	**SK2 SS 45**
1	3.95 ± 0.12ab	3.6 ± 0.23a	3.93 ± 0.09a	3.65 ± 0.22a
3	4.28 ± 0.21abc	3.72 ± 0.22a	4.13 ± 0.04a	4.65 ± 0.10b
5	3.87 ± 0.06a	4.5 ± 0.34ab	4.69 ± 0.42a	5.32 ± 0.35b
8	4.62 ± 0.22bc	5.38 ± 0.18b	5.51 ± 0.23a	5.57 ± 0.08b
10	5.64 ± 0.78c	4.78 ± 0.05ab	4.93 ± 0.17a	4.85 ± 0.02b
12	4.96 ± 0.10c	5.15 ± 0.21b	4.66 ± 0.50a	4.95 ± 0.20b
Day	**Al WF 35**	**Al WF 45**	**Al SS 35**	**Al SS 45**
1	2.58 ± 0.04a	2.63 ± 0.17a	4.09 ± 0.40a	3.74 ± 0.44a
3	3.26 ± 0.08a	2.48 ± 0.32a	3.67 ± 0.36a	3.27 ± 0.38a
5	3.05 ± 0.17a	2.98 ± 0.14a	3.79 ± 0.15a	3.87 ± 0.16a
8	3.21 ± 0.36a	2.91 ± 0.17a	3.72 ± 0.26a	3.42 ± 0.19a
10	3.08 ± 0.33a	n.d	4.42 ± 0.46a	4.36 ± 1.16a

Each value is the mean ± standard error, (n=3). Values not sharing a letter are significantly ($p<0.05$) different within columns, within each treatment, analyzed by ANOVA followed by Tukey's HSD post hoc test or analyzed non-parametrically by Kruskall-Wallis followed by Steel-Dwass post hoc test. n.d= not determined

3.3.1.3. Colorimetric Analyses

Colorimetric L* values are used to measure lightness and range from 0 to 100, where 0 is black and 100 is white. Time and product form significantly affected sugar kelp from the February harvest, where the L* values increased over time and were higher for whole fronds than shredded slaw (Table 3.8). Interestingly, time and temperature significantly increased L* values for June harvested sugar kelp, with increased fading for samples stored at higher temperature over time. However, unlike SK, product form did not significantly affect sugar kelp harvested in June. For winged kelp, only time

significantly increased the L* values, indicating fading over time. These results paralleled sensory color, where the scores dropped over time as the panelists noted that the kelp samples were fading in color.

Table 3.8. L* values for sugar kelp February harvest (SK), sugar kelp June harvest (SK2) and winged kelp (Al) during refrigerated storage.

		L*		
Day	**SK WF 35**	**SK WF 45**	**SK SS 35**	**SK SS 45**
3	17.02 ± 2.29a	15.62 ± 1.91a	11.33 ± 0.32a	10.93 ± 1.09a
5	19.68 ± 0.44a	19.00 ± 1.15a	16.60 ± 0.70a	15.77 ± 0.26b
7	19.30 ± 0.38a	18.73 ± 1.03a	19.91 ± 1.63a	19.52 ± 1.22b
Day	**SK2 WF 35**	**SK2 WF 45**	**SK2 SS 35**	**SK2 SS 45**
1	16.51 ± 0.57ab	16.50 ± 0.53a	14.48 ± 0.48a	15.04 ± 0.65a
3	15.55 ± 0.48a	16.88 ± 0.60a	16.43 ± 0.50a	16.97 ± 0.43ab
5	15.74 ± 0.64ab	15.82 ± 1.18a	14.68 ± 0.96a	14.76 ± 0.51a
8	18.78 ± 0.85b	17.24 ± 0.57a	16.20 ± 0.94a	18.58 ± 0.68b
10	14.01 ± 0.42a	17.98 ± 0.39a	15.81 ± 0.50a	17.27 ± 0.91ab
12	15.16 ± 0.90a	18.10 ± 1.18a	17.36 ± 0.44a	18.29 ± 0.74b
Day	**Al WF 35**	**Al WF 45**	**Al SS 35**	**Al SS 45**
1	16.80 ± 0.30a	16.14 ± 1.36a	14.74 ± 0.50a	13.25 ± 0.54a
3	17.27 ± 2.09a	18.33 ± 1.41a	n.d	n.d
5	15.36 ± 0.31a	17.10 ± 1.43a	13.95 ± 0.43a	14.57 ± 0.26a
8	17.34 ± 2.45a	19.38 ± 1.58a	17.97 ± 0.35b	20.30 ± 0.42b
10	17.52 ± 0.92a	n.d	17.55 ± 0.55b	19.68 ± 0.13b

Each value is the mean ± standard error, (n=3). Values not sharing a letter are significantly ($p<0.05$) different within columns, within each treatment, analyzed by ANOVA followed by Tukey's HSD post hoc test or analyzed non-parametrically by Kruskall-Wallis followed by Steel-Dwass post hoc test. n.d=not determined

The a* values are a measure of redness. For the February sugar kelp, the a* values significantly decreased over time but were not significantly different between the two temperatures (Table 3.9). Although the red color of brown sea vegetables was not apparent to the naked eye, the decrease in these values indicated that the samples were

fading with respect to the red color. Time, temperature and product form significantly affected a* values of the June sugar kelp harvest. For the SK2 whole fronds, no changes in redness values were observed at 35 ºF whereas the values significantly decreased on day 12 at 45ºF. For SK2 shredded slaw, a* values decreased quickly at 45 ºF compared to 35 ºF, indicating that the higher temperature accelerated loss of color. Surprisingly, color values did not significantly change for winged kelp over time or for different treatments, which is contrary to the sensory color scores. This indicates that for winged kelp, the fading as perceived by naked eye was not due to loss of red color over time.

Table 3.9. a* values for sugar kelp February harvest (SK), sugar kelp June harvest (SK2) and winged kelp (Al) during refrigerated storage.

	a*			
Day	**SK WF 35**	**SK WF 45**	**SK SS 35**	**SK SS 45**
3	2.70 ± 0.46b	2.60 ± 0.46b	3.01 ± 0.30c	2.50 ± 0.11b
5	1.94 ± 0.13ab	1.06 ± 0.23ab	1.83 ± 0.07b	1.25 ± 0.08a
7	1.38 ± 0.07a	0.80 ± 0.35a	0.91 ± 0.03a	0.94 ± 0.04a
Day	**SK2 WF 35**	**SK2 WF 45**	**SK2 SS 35**	**SK2 SS 45**
1	4.92 ± 0.42a	4.72 ± 0.22b	4.82 ± 0.37b	4.31 ± 0.02c
3	4.50 ± 0.49a	4.05 ± 0.16ab	4.25 ± 0.19ab	3.92 ± 0.17bc
5	4.82 ± 0.29a	4.56 ± 0.22b	4.75 ± 0.19ab	3.94 ± 0.22bc
8	4.03 ± 0.28a	3.71 ± 0.37ab	3.91 ± 0.48ab	3.22 ± 0.17ab
10	4.71 ± 0.24a	3.62 ± 0.05ab	3.84 ± 0.33ab	3.29 ± 0.07ab
12	5.18 ± 0.41a	3.09 ± 0.36a	2.34 ± 0.14a	2.89 ± 0.24a
Day	**Al WF 35**	**Al WF 45**	**Al SS 35**	**Al SS 45**
1	3.84 ± 0.24a	3.77 ± 0.20a	3.82 ± 0.32a	4.14 ± 0.21a
3	3.68 ± 0.32a	3.84 ± 0.46a	n.d	n.d
5	4.32 ± 0.18a	3.58 ± 0.13a	3.90 ± 0.05a	3.69 ± 0.06a
8	3.57 ± 0.53a	3.61 ± 0.50a	3.66 ± 0.19a	4.22 ± 0.14a
10	4.50 ± 0.19a	n.d	3.79 ± 0.08a	4.18 ± 0.12a

Each value is the mean ± standard error, (n=3). Values not sharing a letter are significantly ($p<0.05$) different within columns, within each treatment, analyzed by ANOVA followed by Tukey's HSD post hoc test or analyzed non-parametrically by Kruskall-Wallis followed by Steel-Dwass post hoc. n.d=not determined

The b* values measure yellowness of the samples. Surprisingly, time and product form did not significantly affect the b* values for sugar kelp harvested in February. However, the higher storage temperature resulted in higher b* values. The yellowness increased for sugar kelp harvested in June over time but no effects of temperature and product form were observed (Table 3.10). For SK2 shredded slaw, there was no change in yellowness values for samples stored at 35 ºF but they dropped significantly by day 10 for samples stored at 45 ºF compared to day 1. These results were similar to a* values results, indicating that the higher storage temperature led to color deterioration more quickly in sugar kelp. Both time and temperature significantly increased winged kelp b* values. However, the differences over time were most prominent in shredded slaw samples stored at 45 ºF, with the yellowness increasing significantly by day 5 compared to day 1.

Table 3.10. b* values for sugar kelp February harvest (SK), sugar kelp June harvest (SK2) and winged kelp (Al) during refrigerated storage.

	b*			
Day	**SK WF 35**	**SK WF 45**	**SK SS 35**	**SK SS 45**
3	6.96 ± 0.73a	7.31 ± 0.45a	7.93 ± 0.34a	8.00 ± 0.08a
5	5.83 ± 0.62a	8.61 ± 0.80a	6.99 ± 0.62a	10.24 ± 0.15a
7	7.50 ± 0.69a	8.79 ± 1.20a	5.93 ± 1.27a	7.58 ± 0.70a
Day	**SK2 WF 35**	**SK2 WF 45**	**SK2 SS 35**	**SK2 SS 45**
1	9.89 ± 0.96a	9.39 ± 1.06ab	9.38 ± 0.96a	8.25 ± 0.26a
3	8.97 ± 1.70a	7.98 ± 0.72a	8.51 ± 0.47a	7.67 ± 0.20a
5	9.90 ± 1.20a	9.93 ± 0.98ab	10.78 ± 1.32a	9.38 ± 1.37ab
8	8.91 ± 2.03a	7.98 ± 0.85a	9.46 ± 1.35a	11.22 ± 1.03ab
10	9.32 ± 0.45a	12.39 ± 0.49bc	10.75 ± 0.70a	12.58 ± 0.89b
12	10.88 ± 0.40a	14.43 ± 1.17c	11.62 ± 0.11a	13.03 ± 0.24b
Day	**Al WF 35**	**Al WF 45**	**Al SS 35**	**Al SS 45**
1	9.18 ± 0.98a	8.95 ± 0.90a	8.27 ± 0.73a	9.12 ± 0.73a
3	7.56 ± 0.58a	8.88 ± 1.11a	n.d	n.d
5	10.87 ± 0.29a	11.18 ± 0.54a	12.12 ± 0.15a	12.95 ± 0.30b
8	10.08 ± 3.12a	12.48 ± 2.06a	12.08 ± 0.76a	15.07 ± 1.16b
10	12.72 ± 0.37a	n.d	13.15 ± 0.21a	15.30 ± 0.25b

Each value is the mean ± standard error, (n=3). Values not sharing a letter are significantly ($p<0.05$) different within columns, within each treatment, analyzed by ANOVA followed by Tukey's HSD post hoc test or analyzed non-parametrically by Kruskall-Wallis followed by Steel-Dwass post hoc. n.d= not determined

Overall, the L*, a*, b* color values provided crucial information on how the color quality deteriorated over time. The fading, which was likely due to loss of pigments such as fucoxanthin and chlorophyll *c*, was captured by increased lightness and yellowness, and decreased redness values. The initial colors between species and harvest seasons varied, with the color of sugar kelp harvested in February being darker.

3.3.1.4. Texture Analyses

Temperature had a significant effect on texture of sugar kelp harvested in February, with much lower TPA force values for samples stored at 45 ºF (Table 3.11). Based on personal observation and sensory scores, the tissues of SK whole fronds at 45 ºF had already softened by day 5, further deteriorating and becoming extremely soft by the end of the study. Although 35 ºF maintained the texture better, by the end of the study, the TPA force values dropped by over 78% compared to day 4. Shredded slaw texture values were highly variable, not following any particular trend. Similarly, the overall force and hardness values for sugar kelp harvested in June were highly variable. However, hardness values increased significantly by day 4 for SK2 shredded slaw stored at 45 ºF. In general, there was a lot of textural variability within and between fronds. Decreases in instrumental texture values were observed over time in selected cases but better methods need to be developed to more robustly quantify changes. In sea vegetable texture, when measuring shear force in *Gracilaria*, other researchers (Paull and Chen 2008) discussed similar challenges in quantifying textural changes during storage.

Table 3.11. TPA force (whole fronds) and compression hardness (shredded slaw) for sugar kelp February harvest (SK) and sugar kelp June harvest (SK2) during refrigerated storage.

	Force (N)		Hardness (N)	
Day	**SK WF 35**	**SK WF 45**	**SK SS 35**	**SK SS 45**
4	76.69 ± 44.02a	5.33 ± 1.87a	12.93 ± 2.79	14.63 ± 1.54b
6	32.82 ± 12.78a	6.02 ± 2.19a	20.76 ± 10.71	6.43 ± 1.10a
8	16.95 ± 8.53a	2.76 ± 1.22a	17.05 ± 1.93	10.68 ± 1.72ab
Day	**SK2 WF 35**	**SK2 WF 45**	**SK2 SS 35**	**SK2 SS 45**
2	20.00 ± 1.57ab	21.96 ± 7.23a	4.80 ± 1.31a	2.77 ± 0.70a
4	54.29 ± 12.30ab	40.85 ± 14.63a	6.08 ± 0.76a	7.03 ± 1.49b
6	60.41 ± 22.35ab	60.17 ± 34.12a	4.97 ± 0.36a	8.38 ± 1.83b
9	60.78 ± 12.96b	29.64 ± 16.70a	9.37 ± 1.76a	9.97 ± 1.86b
11	21.40 ± 8.52ab	28.64 ± 11.17a	6.79 ± 0.99a	8.01 ± 1.13b
13	15.29 ± 1.37a	42.20 ± 10.14a	6.68 ± 1.40a	10.68 ± 0.33b

Each value is the mean ± standard error, (n=3). Values not sharing a letter are significantly ($p<0.05$) different within columns, within each treatment, analyzed by ANOVA followed by Tukey's HSD post hoc test or analyzed non-parametrically by Kruskall-Wallis followed by Steel-Dwass post hoc.

Time significantly increased the shear force and compression hardness values for winged kelp, with shear force values for whole fronds increasing from approximately 5 to 14 N by the end of the study (Table 3.12). This increase in shear force values indicate that the samples were becoming chewier towards the end versus crisp or "easy to snap" in the beginning of the study. For shredded slaw, hardness values increased significantly by day 6 at 35 ºF whereas they significantly increased by day 4 at 45 ºF, indicating that the quality was deteriorating faster at the higher temperature. While measuring whole fronds texture using a kraft knife shear method, it was observed that the sample stuck to the blade a few times as it lost its crisp texture over time. This could be avoided in future studies by holding the sample in place by placing weights on the frond section under investigation.

Table 3.12. Knife blade shear force (only whole fronds) and compression hardness (only shredded slaw) for winged kelp (Al) during refrigerated storage.

	Shear Force (N)		Hardness (N)	
Day	**Al WF 35**	**Al WF 45**	**Al SS 35**	**Al SS 45**
2	5.74 ± 0.50a	5.24 ± 0.48a	5.43 ± 0.86a	5.33 ± 1.43a
4	5.61 ± 0.91a	7.12 ± 0.17a	9.13 ± 1.27a	35.42 ± 14.02b
6	11.53 ± 4.02a	12.01 ± 2.93a	21.74 ± 0.96b	19.49 ± 2.59b
9	12.81 ± 2.31a	14.72 ± 2.35a	25.33 ± 3.19b	30.02 ± 1.84b
11	11.15 ± 2.85a	n.d	19.71 ± 3.79b	16.77 ± 1.84b

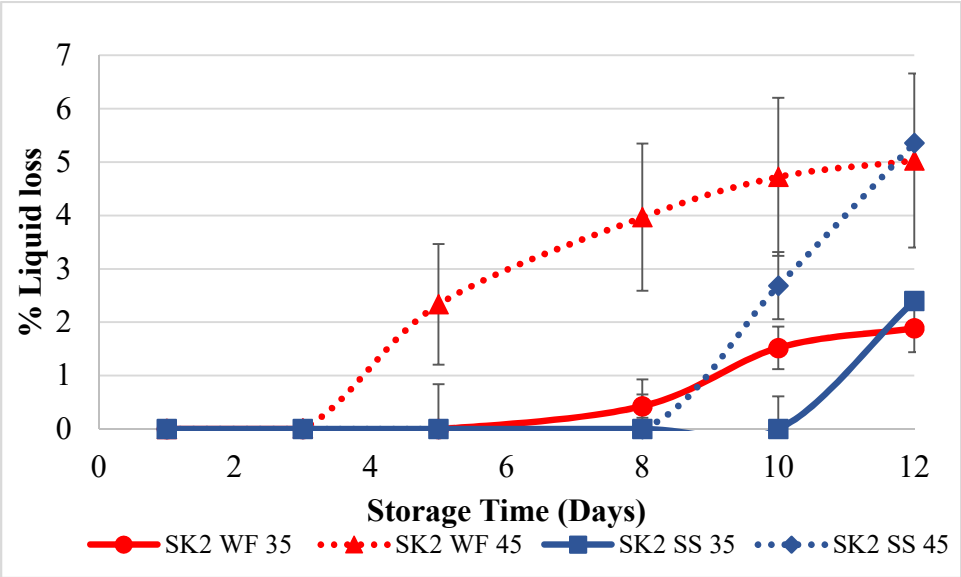
Each value is the mean ± standard error, (n=3). Values not sharing a letter are significantly ($p<0.05$) different within columns, analyzed by ANOVA followed by Tukey's HSD post hoc test or analyzed non-parametrically by Kruskall-Wallis followed by Steel-Dwass post hoc. n.d= not determined

3.3.1.5. Drip Loss

During the sugar kelp (February) shelf life study, there was a noticeable amount of pooled liquid in the sample bags. Cellular liquid loss has been reported as one of the major cause of postharvest deterioration in fresh vegetables, especially highly perishable leafy vegetables (Kader 2002, Toivonen 2011). Quantifying this liquid loss is important not only for assessing quality loss but also for creating proper methods to distribute fresh sea vegetables. Overall, time, temperature and product form significantly affected drip loss in sugar kelp, with an increase in liquid loss over time. It was hypothesized that the shredded slaw would have more liquid loss compared to whole fronds due to cell rupture as a result of chopping. However, the exact opposite was found for sugar kelp during the course of the study, which was unexpected (Fig 3.5). By the end of the study, SK2 whole fronds and shredded slaw samples stored at 45 ºF had approximately 5% liquid loss compared to approximately 2% liquid loss at 35 ºF, indicating that the lower storage temperature maintained better cellular integrity of the samples than the higher storage temperature. However, surprisingly these differences in drip loss were not reflected by

the TPA force values for sugar kelp harvested in June, where no significant effect of temperature was observed.

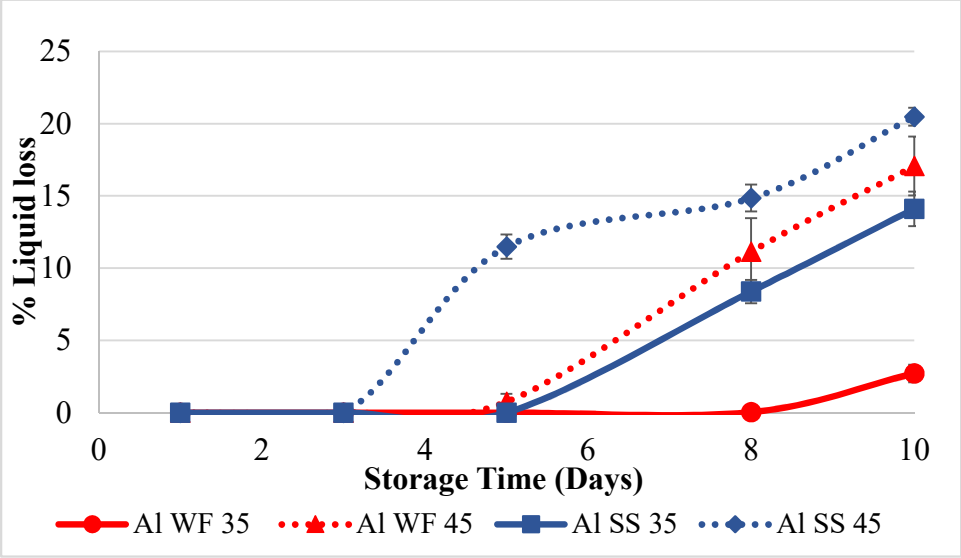
Figure 3.5. Drip loss for sugar kelp harvested in June during refrigerated storage.



Each value represents the mean ± standard error, (n=3).

Similar to SK2, drip loss in winged kelp was significantly ($p<0.0001$) affected by time, temperature and product form. Drip loss in samples stored at 45 ºF started prior to drip loss in the samples stored at 35 ºF, clearly indicating that the higher storage temperature contributed largely to cellular damage leading to loss of cellular liquid (Fig 3.6). For whole fronds, samples stored at 45 ºF lost 17% of liquid compared to merely 2.7% liquid loss for samples at 35 ºF. On day 5, winged kelp shredded slaw stored at 45 ºF had 11.5% liquid loss compared to no drip loss for samples stored at 35 ºF.

Figure 3.6. Drip loss for winged kelp during refrigerated storage.



Each value represents the mean ± standard error, (n=3).

Drip loss proved to be an extremely useful parameter to assess quality changes over time in fresh sea vegetables. Moreover, evident changes in texture and appearance could be related to loss of cellular liquid. As time progressed, the samples lost their crispiness and became wilted which was further confirmed by poor sensory texture scores for the kelp species towards the end of the study.

3.3.1.6. Soluble Protein

The soluble protein content was variable among species and between treatments. Sugar kelp harvested in February had extremely low soluble protein compared to SK2 and Al (Table 3.13). On the contrary, Schiener and others (2015) reported higher crude protein for sugar kelp harvested in winter than summer. However, soluble protein measures only the water soluble proteins whereas crude protein typically measures all the nitrogenous compounds in the sample. For winged kelp, soluble protein content for whole fronds stored at 35 ºF dropped significantly by day 5 but although the soluble protein content dropped, it was not significantly lower over time at 45 ºF. Soluble protein content

for winged kelp shredded slaw at 35 ºF dropped significantly by day 5 whereas it dropped by day 3 for samples at 45 ºF. These results indicate that for winged kelp slaw, higher temperature accelerated loss of soluble protein, contributing to overall quality loss. This decrease in soluble protein could be related to increased drip loss over time. However, given the highly variable results, it appears that soluble protein was not a reliable indicator of quality loss in the brown sea vegetables studied.

Table 3.13. Soluble protein (mg/g wet weight) for sugar kelp February harvest (SK), sugar kelp June harvest (SK2) and winged kelp (Al) during refrigerated storage.

Day	**SK WF 35**	**SK WF 45**	**SK SS 35**	**SK SS 45**
1	n.d	0.93 ± 0.35a	1.24 ± 0.53a	1.61 ± 0.73a
3	2.38 ± 0.86a	0.97 ± 0.30a	1.51 ± 0.45a	0.65 ± 0.23a
5	1.25 ± 0.46a	0.87 ± 0.46a	0.73 ± 0.36a	1.35 ± 0.61a
7	0.87 ± 0.10a	0.36 ± 0.01a	0.80 ± 0.32a	0.35 ± 0.01a
Day	**SK2 WF 35**	**SK2 WF 45**	**SK2 SS 35**	**SK2 SS 45**
1	11.20 ± 1.88a	10.96 ± 4.31a	8.24 ± 1.96a	13.69 ± 0.81abc
3	7.43 ± 3.84a	9.85 ± 6.88a	4.66 ± 1.21a	14.87 ± 2.52bc
5	15.26 ± 5.66a	11.65 ± 5.78a	16.93 ± 1.66a	16.77 ± 2.03c
8	22.92 ± 3.21a	12.98 ± 3.57a	11.83 ± 4.77a	11.76 ± 2.65abc
10	4.37 ± 0.37a	2.85 ± 0.91a	3.90 ± 0.08a	3.78 ± 1.17a
12	11.25 ± 4.68a	5.69 ± 0.65a	7.51 ± 2.89a	6.64 ± 2.71ab
Day	**Al WF 35**	**Al WF 45**	**Al SS 35**	**Al SS 45**
1	11.33 ± 2.91b	10.54 ± 4.48a	13.18 ± 0.67c	15.22 ± 3.02b
3	6.11 ± 2.34ab	8.98 ± 0.27a	10.08 ± 1.27c	7.53 ± 0.90a
5	2.55 ± 0.60a	4.45 ± 0.79a	2.84 ± 0.46ab	6.57 ± 1.60a
8	6.20 ± 1.72ab	4.04 ± 1.16a	6.03 ± 1.12b	8.06 ± 0.72ab
10	0.62 ± 0.09a	n.d	1.43 ± 0.33a	1.84 ± 0.44a

Each value is the mean ± standard error, (n=3). Values not sharing a letter are significantly ($p<0.05$) different within columns, within each treatment, analyzed by ANOVA followed by Tukey's HSD post hoc test or analyzed non-parametrically by Kruskall-Wallis followed by Steel-Dwass post hoc. n.d=not determined.

3.3.1.7. Total Volatile Base Nitrogen

Total volatile base nitrogen (TVBN) is an indirect method to assess microbial spoilage in muscle foods and is often used to assess quality loss is seafood. It measures the amount of volatile nitrogenous compounds including trimethylamine, ammonia and methylmercaptan, which may be produced by the bacteria present in the sample from non-protein nitrogen (Gram and Huss 1996). Previous authors have reported that sea vegetables contain trimethylamine, methylamine and ammonia (Smith and Young 1953, Mouritsen and others 2013). Therefore, TVBN was determined in sugar kelp harvested in June and in winged kelp. The TVBN values for SK2 were extremely low throughout the study whereas the values were higher for winged kelp, in comparison (Table 3.14). However, there was no effect of time, temperature or product form for either species. The low TVBN values may be related to the low microbial activity in these species and the low nitrogenous content compared to fish and shellfish.

Table 3.14. TVBN (mg N/100g), sugar kelp June harvest (SK2) and winged kelp (Al) during refrigerated storage.

Day	SK2 WF 35	SK2 WF 45	SK2 SS 35	SK2 SS 45
1	0.34 ± 0.34a	0.45 ± 0.30a	0.45 ± 0.45a	n.d
3	0.68 ± 0.52 a	n.d	0.90 ± 0.49a	0.11 ± 0.11a
5	n.d	n.d	0.34 ± 0.34a	n.d
8	1.13 ± 0.45a	0.23 ± 0.23a	0.23 ± 0.23a	0.45 ± 0.23a
10	0.45 ± 0.23a	0.45 ± 0.23a	0.68 ± 0.00a	0.68 ± 0.00a
12	0.45 ± 0.23a	0.90 ± 0.23a	0.90 ± 0.23a	0.90 ± 0.23a
Day	**Al WF 35**	**Al WF 45**	**Al SS 35**	**Al SS 45**
1	3.78 ± 0.22a	4.01 ± 0.77a	3.34 ± 0.39a	2.67 ± 0.00a
3	3.34 ± 0.00a	4.01 ± 0.39a	2.89 ± 0.45a	3.12 ± 0.22a
5	4.67 ± 1.54a	4.45 ± 0.80a	3.34 ± 0.00a	3.78 ± 0.45a
8	5.34 ± 1.39a	4.67 ± 0.39a	3.34 ± 0.00a	3.34 ± 0.77a
10	5.34 ± 1.39a	n.d	3.34 ± 0.77a	2.23 ± 0.22a

Each value is the mean ± standard error, (n=3). Values not sharing a letter are significantly ($p<0.05$) different within columns, within each treatment, analyzed by ANOVA followed by Tukey's HSD post hoc test or analyzed non-parametrically by Kruskall-Wallis followed by Steel-Dwass post hoc. n.d=not detected

3.3.2. Nutritional Analyses

The moisture content of sea vegetables typically ranges from 80 g/100g to 90 g/100g fresh sample (MacArtain and others 2007). The moisture content for both the kelps, 90.2 g/100g for sugar kelp and 86.5 g/100g for winged kelp, were within the typical range (Table 3.15). Schiener and others (2015) reported 84.9 and 85.5 g/100g moisture content of sugar kelp and winged kelp, respectively. In general, the ash content of sea vegetables can range from anywhere between 8 g/100g to 55 g/100g (dwb) (Ito and Hori 1989, Rupérez 2002, McDermid and Stuercke 2003, Baghel and others 2014). The yearly average ash content reported was 31.7 and 25.3 g/100g for sugar kelp and winged kelp, respectively (Schiener and others 2015). However, these values were lower than ash values found for both the kelps in this study. It is noteworthy, though, that the values

reported by Schiener and others (2015) were averages of different harvests throughout the year. The crude fat content for both the brown sea vegetables was below 4 g/100g. Crude fat content of sea vegetables is generally low, approximately 1-4 g/100g (dwb) (McDermid and Stuercke 2003, Hong and others 2007, Rohani-Ghadikolaei and others 2012).

Table 3.15. Proximate analyses of fresh sugar kelp and winged kelp (g/100g, dwb) unless specified otherwise.

Species	Moisture (wwb)	Ash	Fat	Protein	Carbohydrate (by difference)
Sugar kelp	90.2 ± 0.1	41.7 ± 0.9	3.0 ± 0.1	19.9 ± 0.1	35.3
Winged kelp	86.5 ± 0.3	31.3 ± 1.3	2.4 ± 0.1	8.3 ± 0.1	58.4

Each value represents the mean ± standard deviation of pooled samples, analyzed in triplicate, except for protein which was analyzed in duplicate.

In general, brown sea vegetables have lower protein content compared to red and green sea vegetables, (Mabeau and Fleurence 1993, Galland-Irmouli and others 1999, Burtin 2003, Misurcova 2011, Patarra and others 2011). In the current study, crude protein content for sugar kelp was 19.9 g/100g and 8.3 g/100g for winged kelp. However, lower protein content for sugar kelp and higher protein content for winged kelp was previously reported by others (Schiener and others 2015). The carbohydrate content was calculated by subtracting the average values for other major food components and hence, does not have standard deviation. The carbohydrate content of sea vegetables, which are rich in dietary fiber, can range from 33 to 75 g/100g (dwb) (Bocanegra and others 2009). Carbohydrate content of both the kelps fell in this range. However, these values are lower

than carbohydrate content reported in sugar kelp and winged kelp by Schiener and others (2015).

Table 3.16. Selected minerals of fresh sugar kelp and winged kelp (mg/100g, dwb).

Selected Mineral	Sugar kelp	Winged kelp
Calcium	620.2 ± 6.5	895.8 ± 33.8
Potassium	1,3951.3 ± 235.6	7,530.4 ± 279.0
Magnesium	662.0 ± 5.5	817.2 ± 6.2
Phosphorus	402.2 ± 5.4	245.9 ± 1.1
Aluminum	56.6 ± 5.7	22.0 ± 0.64
Copper	0.0 ± 0.0	0.0 ± 0.0
Iron	32.2 ± 5.7	49.6 ± 2.8
Sodium	4,382.8 ± 149.7	4,868.6 ± 153.0
Zinc	1.7 ± 0.9	1.6 ± 0.4

Each value represents the mean ± standard deviation of pooled samples, analyzed in triplicate.

Both the species contained minerals commonly found in sea vegetables (Table 3.16) (Rupérez 2002, MacArtain and others 2007, Rao and others 2007, Astorga-España and others 2015). Sugar kelp had higher levels of potassium, phosphorus, aluminum and zinc compared to winged kelp. Both the kelps were rich in potassium and did not contain measurable levels of copper. However, low levels (~0.2-0.5 mg/100g) of copper were reported in sugar kelp and winged kelp by Schiener and others (2015).

Vitamin C levels were also assessed in this study. Selected sea vegetables including dulse are considered good sources of vitamin C. The vitamin C content of sugar kelp and winged kelp were 31.4 ± 0.2 and 20.7 ± 0.5 mg/100g fresh sample, respectively. McDermid and others (2003) reported that no vitamin C was detected in the two brown sea vegetables they assessed. However, MacArtin and others (2007) reported vitamin C

content of *Laminaria spp.* to be 35 g/100g fresh weight, which is close to the vitamin C content of sugar kelp. In this study differences in the vitamin C content could be due to differences in harvest season, location and species.

3.4. Conclusions

This is the first study reporting the refrigerated shelf life of fresh, farm-raised brown sea vegetables. The promising results of this study may help bolster the growth of the aquaculture industry in New England. The results of this study indicate that based primarily on sensory evaluation, for sugar kelp harvested in February, a 6-day acceptable quality shelf life was achieved for whole fronds whereas a 7-day shelf life was achieved for shredded slaw for samples stored at 35 ºF. Surprisingly, harvest season had a huge impact on shelf life of sugar kelp, as a 12-day acceptable quality shelf life was achieved at 35 ºF for whole fronds and shredded slaw of sugar kelp harvested in June. Both, winged kelp whole fronds and shredded slaw, had an acceptable quality shelf life of 8 days at 35 ºF. For both kelps, the higher storage temperature reduced the shelf life. Drip loss, in both species, contributed to quality deterioration immensely, further impacting texture and appearance of the product. Drip loss may have a large impact on sales of these fresh sea vegetables. Microbial activity was variable and may have contributed to quality loss in some cases. However, quality loss linked to physical deterioration was the primary cause for loss of acceptability of these sea vegetables.

Overall, both the brown sea vegetables were nutrient-dense and had nutrient profiles similar to those previously reported wild harvested forms in the literature. This information could be used by the sea vegetable distributors to attract consumers interested in sustainably sourced foods that are high in nutritional value. They were high

in total minerals including potassium, calcium and magnesium, and low in lipid content, making these kelps attractive to health conscious consumers. Winged kelp was high in carbohydrate content and low in protein content while sugar kelp was lower in carbohydrate but higher in protein content.

CHAPTER 4

EFFECTS OF BLANCHING AND FREEZING ON ANTIOXIDANT CAPACITY OF DULSE (*Palmaria palmata)*, *Gracilaria tikvahiae,* SUGAR KELP (*Saccharina latissima)* AND WINGED KELP (*Alaria esculenta*)

4.1. Justification and Objectives

A lot of attention has been given to analyzing antioxidants present in sea vegetables over the past decade. Claims such as "high in antioxidants" have been shown to affect consumers' attitudes about product quality positively, and may be beneficial in promoting farm-raised sea vegetables (Daniells 2009). In a recent article about the top ten food trends in North America, the authors cited multiple trend reports showing that 30% of consumers made a strong effort to consume more minimally processed foods (Sloan 2015). Another article reported that approximately 55-60% of consumers are likely to buy or continue purchasing a product having an antioxidant claim (Daniells 2009). Most of the research reported to date on bioactive compounds in sea vegetables has been on dried, wild harvested product. However, sea vegetables contain heat sensitive nutrients such as vitamins C and phenolic compounds which are likely labile to thermal processing. Sea vegetable producers in the New England area have developed minimally processed sea vegetable products including fresh and frozen salads; 'ready to eat/cook' blanched and salted fronds, and frozen prepared soups (Redmond 2012). Blanching of sea vegetables results in attractive green color of the samples, making them more attractive to the American consumers. Additionally, it also aids in inactivation of enzymes that may lead to off-flavor development, change in nutritional quality and texture of the food (Rahman and Perera 2007). However, commonly used processing methods such as

blanching and freezing may negatively affect bioactive compounds present in these value-added products. Previous research has shown that the amount of bioactive compounds present in different parts of sea vegetables, such as the blade versus stipe, may vary (Connan and others 2006). However, to date there have been no reports on the effects of selected processing treatments and source of edible tissue (blade/stipe) on antioxidant capacity of sea vegetables.

The overall goal of this study was to determine the effects of various processing methods on bioactivity of fresh, farm-raised, sea vegetables. The specific objectives included assessing effects of blanching, freezing and short term frozen storage on the total phenolic content (TPC) and antioxidant capacity (using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and ferric reducing antioxidant power (FRAP) assays) of two red (*Gracilaria tikvahiae* (Gr) and *Palmaria palmata* (Du)), and two brown (*Saccharina latissima* (SK) and *Alaria esculenta* (Al)) freshly harvested sea vegetables. For the evaluation of brown sea vegetables (kelps), an additional objective was to determine differences in bioactivity, if any, between the blades and stipes.

4.2. Materials and Methods

4.2.1. Experimental Design

Four processing treatments were chosen for this study for all the four sea vegetables: fresh, blanched, fresh frozen and blanched frozen (Table 4.1). The brown sea vegetables were sorted into blades (WF) and stipes (ST) and processed similarly. Samples were blanched at 80 ºC for 1 min and the frozen treatments were stored at -20 ºC for one month. All the processing was done in triplicate (A, B, C).

Table 4.1. Experimental treatments and codes

Treatment Code	Blanched	Frozen
Fr (Control)		
FF		X
BL	X	
BF	X	X

4.2.2. Determining Blanching Parameters

Low-temperature long-time (80 ºC for 1 min) and high-temperature short-time (100 ºC for 5 s) treatments were selected for preliminary testing. Final blanching temperature and duration were chosen based on a sensory evaluation of a *Gracilaria* salad made with blanched *Gracilaria* from both the treatments. *Gracilaria* was selected for sensory evaluation due to its availability. At test time, a triangle test followed by a preference test was conducted to assess whether panelists could differentiate between treatments, and if so, which one of the two treatments they preferred. Approval for research with human subjects was obtained from the Institutional Review Board (IRB) prior to conducting sensory analyses. The panelists were provided with an informed consent (Appendix H) and $5 for compensation.

4.2.2.1. Sample Processing for Sensory Evaluation

Fresh *Gracilaria*, was harvested in October from Clark Clove farm (Bristol, Maine) and delivered in a cooler on the same day. Due to poor growth of farm-raised *Gracilaria*, wild harvest was used instead. *Gracilaria* was washed under cold tap water to remove any dirt and then dried with paper towels. Blanching took place in the School of

Food and Agriculture's commercial kitchen. Two pots were filled with 10 liters of tap water each. Five hundred grams of *Gracilaria* were added to the water once the desired temperature was reached, 80 ºC or 100 ºC, and kept in the water for 60 s or 5 s, respectively. The temperature of the water was monitored with a thermocouple (Omega, Stamford, CT). After blanching, *Gracilaria* was put in a strainer, and then added to an ice water bath, which had equal proportions of water and crushed ice (1:1), for one minute. The sample was strained again and then spun in a salad spinner for 1 minute to remove excess water.

4.2.2.2. Salad Preparation

An Asian salad dressing was made using ingredients from a local supermarket (Hannaford, Old Town, ME) one day prior to the sample delivery and then refrigerated overnight (Table 4.2). All of the ingredients were mixed together in a salad bowl by hand using a whisk. The same dressing was used for both the treatments the next day.

Table 4.2. Salad dressing formulation

Ingredient	**Amount (g)**	**% Weight**
Rice Vinegar	380	36.3
Sugar	240	22.9
Soy Sauce	150	14.3
Sesame Oil	120	11.5
Lime Juice (bottled)	100	9.6
Grated Ginger (fresh)	57	5.4
Total	1047	100

Salads were prepared using blanched *Gracilaria*, shredded carrots, salad dressing and toasted sesame seeds (Table 4.3). Both the salads were thoroughly mixed so that the ingredients were well-dispersed. The salads were allowed to chill in the refrigerator, and taken out of the refrigerator 15 minutes prior to the sensory evaluation.

Table 4.3. Salad formulation

Ingredient	**Amount (g)**	**% Weight**
Gracilaria	250	49.2
Shredded Carrot	127	25
Salad Dressing	125	24.6
Sesame Seeds	6	1.2
Total	508	100.0

4.2.2.3. Sensory Evaluation

A triangle test was chosen to determine if consumers could differentiate between the two products. In this test, panelists were presented with three samples in a randomized order, of which two samples were identical. The panelists had to choose the odd/different sample (Meilgaard and others 2006). Twelve panelists familiar with sea vegetables were recruited via word of mouth from University of Maine to participate in the test. The panelists were briefed on what a triangle test is and how to address the question. The test took place under normal white light. A tray with three paper cups filled with 20 g of salad each and a paper evaluation ballot (Appendix I and J) was prepared for each panelist. Each cup had a unique three-digit code, and water was provided to clean their palate. A fork and napkin were provided, and a maximum of four panelists at a time

were allowed in the room at once to give them enough space and attention, if needed. After panelists chose the odd sample, that sample cup was removed from the tray and the panelists were requested to continue with the preference test. The number of correct responses were counted and compared to the tables for the critical number of correct responses for statistical significance (Meilgaard and others 2006).

4.2.3. Sample Processing for Antioxidant Assays

Processing of all the four species took place on separate days, depending on their harvesting season. *Gracilaria* was harvested in November, 2015 whereas dulse, winged kelp, and sugar kelp were all harvested in April, 2016. Except for *Gracilaria*, all the other species were farm-raised. The samples were harvested from Clark Cove farm (Bristol, ME), shipped in a cooler overnight, and processed within 2 days of the harvest. The samples were washed under cold tap water to remove any dirt and degraded samples, and were then patted dry with paper towels. During the processing, all the samples were kept cold on ice in a cooler lined with plastic trays to avoid any chilling injury. The two brown species, sugar kelp and winged kelp, had an additional step before the treatments were further processed. Samples were cut by hand to separate the blades and stipes prior to blanching or freezing. All the blades and stipes were mixed within species to insure homogeneity. Two hundred and twenty-five grams of sample were processed for each species and plant part in triplicate for all the treatments except sugar kelp stipes. A lesser amount (150g) was used for sugar kelp stipes due to a shortage of the harvested sample.

4.2.3.1. Fresh

Fresh, unprocessed samples, were randomly selected prior to being weighed and packaged in pre-labelled polyethylene bags (Ultrasource, Kansas, MO). These bags were heat sealed after pressing out the air by hand.

4.2.3.2. Blanching

Multiple pots were filled with tap water and brought up to the required temperature. The sample was added to the water at a ratio of 1:20 (w/v). The temperature of the water was monitored with a thermocouple (Omega, Stamford, CT). The sample was added to the hot water and transferred to a strainer after 60 s. The sample was then added to a 4-liter ice bath, which had equal proportion of water and crushed ice (1:1) for one minute. The sample was strained again and then spun in a salad spinner for 1 minute. Blanched samples were reweighed and packaged in plastic bags. The bags were heat sealed after air was removed manually.

4.2.3.3 Blast Freezing

All the samples were blast frozen (Southeast Cooler, Lithia Springs, GA) at -30 ºC post processing for 1 h. These were then either prepared for freeze-drying (VirTis Ultra, Warminster, PA) or frozen storage. The FF and BF samples were transferred to the freezer and the Fr and BL samples were freeze-dried immediately. The freeze drying cycle was for 20 h but multiple cycles were used until the samples reached a constant weight. The freeze-dried samples were crushed and stored in whirlpack bags at -80 ºC (VWR International, Radnor, PA) until further analysis. One week prior to the analyses, all the samples were ground using a coffee grinder and stored at -80 ºC until extraction.

4.2.3.4. Frozen Storage

For FF and BF treatments, samples were packaged as described in 4.3.2 and 4.3.3. These samples were stored at -20 ºC in a walk-in freezer in the Matthew Highland's Pilot Plant (Orono, ME) for one month. The temperature was chosen based on what industry would use to store their frozen samples.

4.2.4. Reagents

All reagents were purchased from Fisher Scientific (Waltham, MA) unless otherwise noted.

4.2.5. Preparation of Sample Extract

Ground, freeze-dried samples (2.00 ± 0.005 g) were extracted with 20 mL 60% methanol for 24 h on an orbital shaker (Fisher Scientific, Waltham, MA) at 210 rpm. Next, the samples were centrifuged at 2100 xg (Beckman Avanti J-25, Brea, CA) for 10 minutes. The supernatant was collected and a pellet wash was performed twice by adding 10 mL of 60% methanol, shaking for 10 minutes on the shaker, and then centrifuging. All the supernatant was pooled, then brought to 50 mL with distilled water, and then vortexed for 30 s to insure to adequate mixing. The 24 h extraction time and 60% methanol concentration for extraction of polyphenols were chosen based on preliminary tests to maximize extraction of polyphenols.

4.2.6. Determination of Total Phenolic Content

Total phenolic content of the sample extract was determined according to the Folin-Ciocalteu method (Taga and others 1984, Matanjun and others 2008, Rajauria and others 2010). One and a half milliliters of Folin-Ciocalteu (Sigma Aldrich, St. Lois, MO) diluted with water (1:10) was added to a 200 µL aliquot of sample extract and vortexed

thoroughly. After 5 min, 1.5 mL of 6% sodium bicarbonate solution was added and vortexed thoroughly. Samples were incubated for 1 h in the dark. Varying concentrations (0-200 µg/mL) of gallic acid were used as a standard. The samples were blanked against 40% methanol because the sample extracts had been diluted with distilled water, resulting in final methanol concentration of 40%. Absorbance was measured at 725 nm using a UV-vis spectrophotometer (Beckman Du 530, Brea, CA). Total phenolic content was expressed as mg gallic acid equivalents per gram of freeze-dried sample. Analyses were run in duplicate and the values were averaged per treatment replicate.

4.2.7. Antioxidant Assays

4.2.7.1. 2, 2-diphenyl-1-picrylhydrazyl (DPPH) Assay

DPPH radical scavenging activity of sample extracts was determined based on Blois (1958) with modifications. DPPH (0.2 mM) (Sigma Aldrich, St. Lois, MO) was prepared in 200 proof ethanol. Fresh solution was prepared each day of analyses. Varying volumes of sample extract (0.5-2 mL) were brought up to 2 mL with 40% methanol. 2 mL of DPPH solution was added to this, mixed thoroughly and incubated for 30 min in the dark. If the samples were too concentrated and their absorbance values were outside the standard curve, then the sample extracts were diluted using distilled water. The samples turned deep purple on addition of DPPH and then turned yellow if the free radical was quenched. Sample blanks were prepared in the same way but 2 mL of 200 proof ethanol instead of 0.2 mM DPPH was added to the sample extracts which were then incubated for 30 min in the dark. The control, 40% methanol, was treated the same way as the sample and sample blank, where either 2 mL DPPH or ethanol was added to 2 mL 40% methanol. The absorbances were all measured against 100% ethanol at 517 nm. The

following formula was used to calculate % inhibition:

$$\% \text{ DPPH inhibition} = \frac{\text{Control Abs} - (\text{Sample Abs} - \text{Sample Blank Abs})}{\text{Control}} \times 100$$

The % inhibition results were plotted against varying concentrations (g/mL) of sample using MS Excel. Linearity was ensured by looking at the R^2 values and EC_{50} was calculated using the slope and constant of the plotted line. The assay was performed in duplicate and the average was expressed as EC_{50} (mg/mL), the concentration of sample necessary for a 50% inhibition of DPPH activity.

4.2.7.2. Ferric Reducing Antioxidant Power (FRAP) Assay

The antioxidant capacity was also assessed according to the method described by Benzie and Strain (1996), with some modifications. The FRAP reagent was prepared fresh daily by mxing 300 mM acetate buffer (pH 3.6), 10 mM 2,4,6-Tripyridyl-s-Triazine (TPTZ) solution and 20 mM $FeCl_3.6H_2O$ solution (100:10:10). This solution was stirred and warmed to 37 ºC in a water bath. An aliquot of 3 mL FRAP reagent was added to 100 µL sample extract or varying concentrations (0-1000 µM) of the $FeSO_4{\cdot}7H_2O$ standard directly in the cuvette. The absorbance was measured at 593 nm after exactly 4 min. The analysis was performed in duplicate and their average was expressed in µmol ferrous sulfate equivalents per gram of freeze-dried sample.

4.2.8. Statistical Analysis

Data were analyzed using JMP 12.2 (SAS Software, Cary, NC). Shapiro-Wilk's normality test and Levene equality of variances were used to assess data prior to further analyses. One-way analysis of variance (ANOVA) was selected to find treatment

differences. Tukey's Honest Significant Difference (HSD) test was selected for post-hoc analyses. A significance level of $p<0.05$ was chosen for all statistical analyses. Pearson correlation between phenolic content and the antioxidant assays was determined to understand their relationships.

4.3. Results and Discussion

While processing, it was observed that the stipes of both the kelps were dissimilar, with sugar kelp stipes being hollow and light-weight whereas winged kelp stipes were solid and thick. However, the inside of both the kelp stipes had a lighter color than the outside, browner color. All the four sea vegetables, irrespective of whether they were rhodophyta or phaeophyta, instantly changed color to green upon blanching. Immediately after blanching, they gave off a distinct odor, however, the odor faded as the sample bags were being prepared. After freeze drying, it was observed that the blanched treatment whole fronds were less dense, and absorbed extraction solvent completely, making them more viscous, compared to the non-blanched samples. The extract color differed depending on species and treatment, with paler colors for blanched treatments.

With regard to the *Gracilaria* sensory test, the panelists could not significantly differentiate between the two blanching treatments during the triangle test, based on the critical number of correct responses required according to Meilgaard and others (2006). The blanching treatment at 80 ºC for 1 min was selected based on two considerations; the sensory evaluation showed us that there were no detectable differences between the two treatments and because this specific treatment has been used previously to blanch sea vegetables (McHugh 2003, Boulom and others 2014).

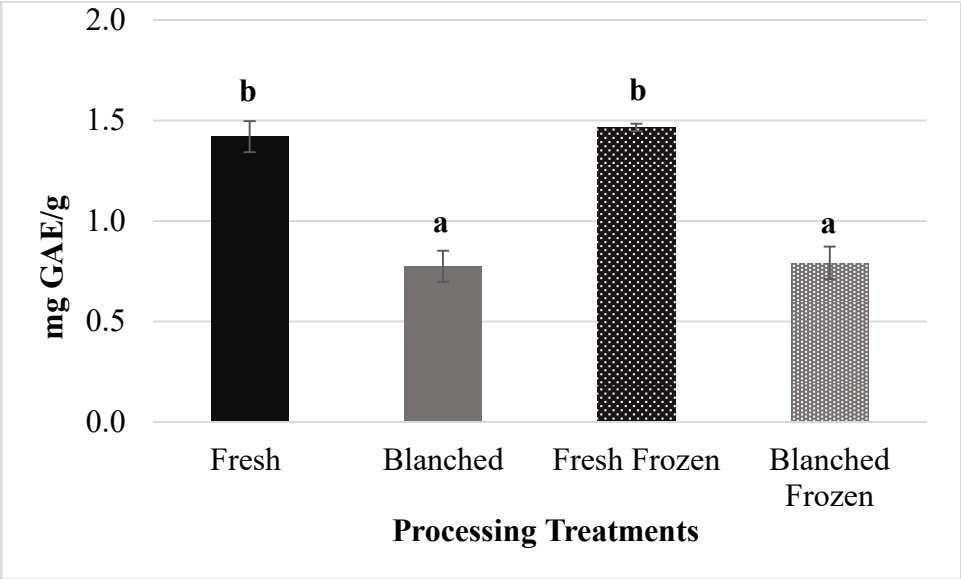
4.3.1 Total Phenolic Content (TPC)

The results of the TPC assays indicate that the blanched samples had significantly ($p<0.01$) lower total phenolic content compared to the fresh and fresh frozen samples for all of the species and plant parts (Fig 4.1- 4.4). The TPC ranged from 1.42 to 17.44 mg GAE/g sample for the fresh and fresh frozen samples and from 0.77 to 7.44 mg GAE/g sample for blanched and the blanched frozen samples, indicating that blanching reduced the TPC by approximately half.

Although blanching reduced the TPC in *Gracilaria* (Fig 4.1) and dulse (Fig 4.2), the effect was larger in *Gracilaria* ($p<0.0001$). In the kelp species, all samples were equally affected by blanching, except for the frozen SK blades, which did not significantly drop in response to blanching (Fig 4.3 – 4.4). The observed decreases in the phenolic content as a result of blanching were likely due to the loss of the highly water soluble phenolic compounds (Cheynier 2012), particularly the ones with lower molecular weight including gallic, gentisic and protocatechuic acid, present in sea vegetables (Sabeena Farvin and Jacobsen 2013). Moreover, blanching may have caused cellular damage or disruption, leading the more complex polyphenols to be released to the blanch water. However, Rajauria and others (2010) reported a 75.6 % increase in TPC in sugar kelp that was hydrothermally processed at 95 ºC for 15 min, explaining that the high temperature and duration could have released previously bound phenolic compounds. Phenolic compounds are often conjugated with sugars and proteins in the intracellular matrix (Randhir 2008) and prolonged hydrothermal treatment could have resulted in disassociation of such bonds (Rajauria and others 2010). In the current study, we found that blanching caused TPC in fresh SK stipes to plummet by over 70%, the highest drop

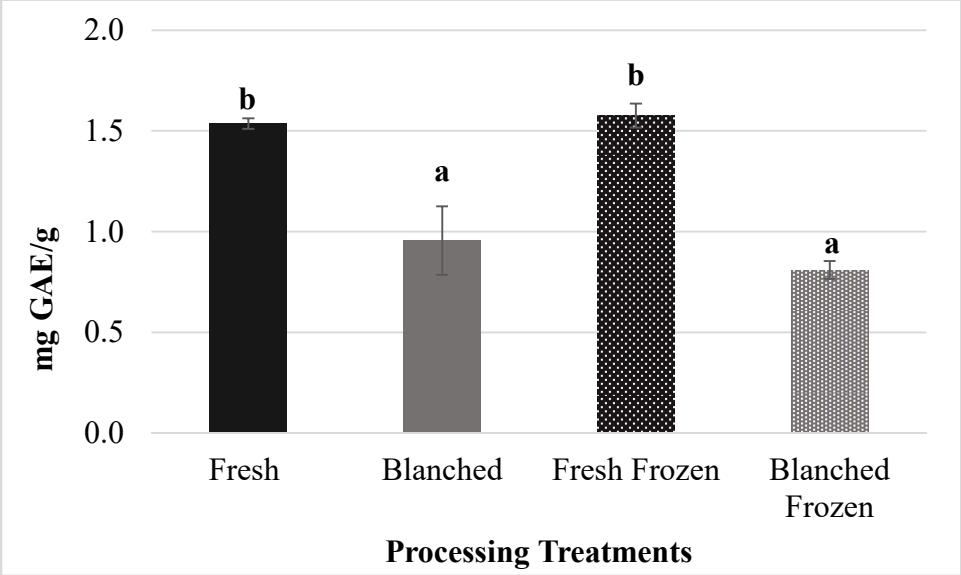
for any of the species and tissues evaluated (Fig 4.3). As previously mentioned, discoloration of red and brown colors in red and brown sea vegetables was observed post blanching, indicating loss of water soluble pigments, which are often polyphenols or their derivatives (Cheynier 2012).

Figure 4.1. Total phenolic content of *Gracilaria*



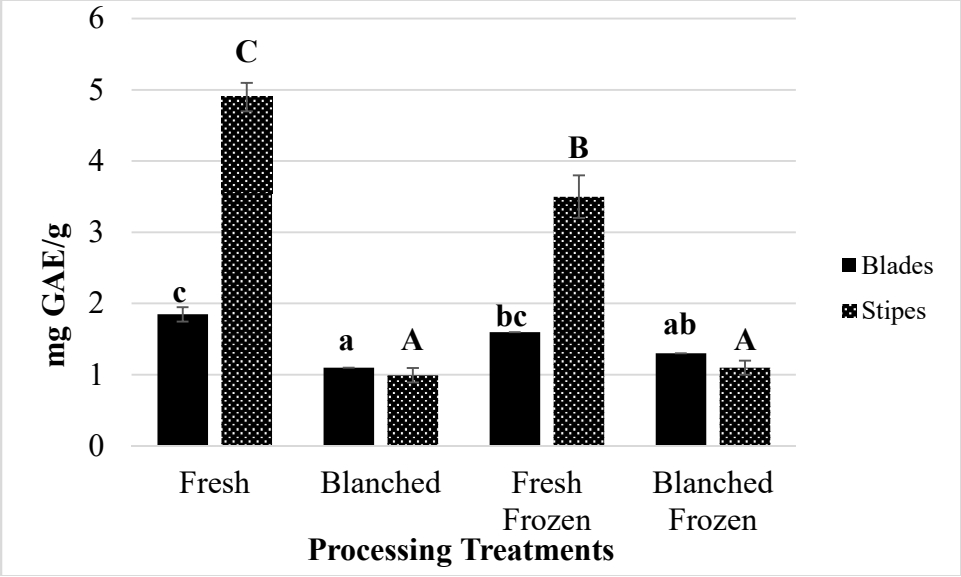
Each value is the mean ± standard deviation, (n=3). Treatments not sharing a letter are significantly ($p<0.05$) different based on one-way ANOVA followed by Tukey's HSD post-hoc. GAE = gallic acid equivalents. GAE = gallic acid equivalents.

Figure 4.2. Total phenolic content of dulse



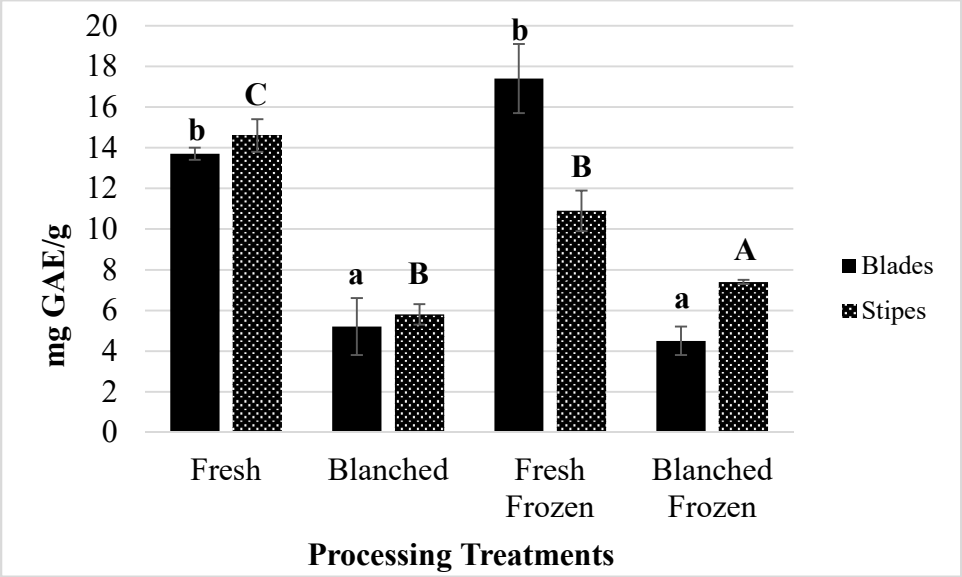
Each value is the mean ± standard deviation, (n=3). Treatments not sharing a letter are significantly ($p<0.05$) different based on one-way ANOVA followed by Tukey's HSD post-hoc. GAE = gallic acid equivalents. GAE = gallic acid equivalents.

Figure 4.3. Total phenolic content of sugar kelp



Each value is the mean ± standard deviation, (n=3). Treatments not sharing a lowercase (blades) or uppercase (stipes) letter are significantly ($p<0.05$) different based on one-way ANOVA followed by Tukey's HSD post-hoc. GAE = gallic acid equivalents.

Figure 4.4. Total phenolic content of winged kelp



Each value is the mean ± standard deviation, (n=3). Treatments not sharing a lowercase (blades) or uppercase (stipes) letter are significantly ($p<0.05$) different based on one-way ANOVA followed by Tukey's HSD post-hoc. GAE = gallic acid equivalents.

In contrast to the effects of blanching, there were negligible differences in TPC due to frozen storage in *Gracilaria* (Fig 4.1), dulse (Fig 4.2), and sugar kelp (Fig 4.3) and winged kelp (Fig 4.4) blades. It is interesting to note, however, that freezing (and frozen storage) significantly ($p<0.0001$) reduced the TPC in the stipes of the brown sea vegetable species (sugar kelp and winged kelp) which was unexpected.

Brown sea vegetables contain a group of polyphenols called phlorotannins that contribute largely to their high antioxidant capacity (Wang and others 2009). They are comprised of phloroglucinol units (Fig 4.5) with up to 8 interconnected rings and 3 hydroxyl groups, which aids their resonance stability as an antioxidant (Koivikko and others 2007, Freile-Pelegrin and Robledo 2013). Their absence in red sea vegetables often results in low antioxidant activity in comparison to brown sea vegetables. In the current study, red sea vegetables (*Gracilaria* and dulse) had lower TPC compared to the

brown sea vegetables. Other authors (Jiménez-Escrig and 2001, García-Casal and others 2009) have reported similar trends when comparing TPC in red and brown sea vegetables.

Figure 4.5. Structure of Phloroglucinol

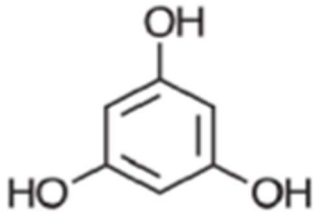


Image from Gupta and Abu-Ghannam (2011)

More recently, researchers have been interested in intra-thallus TPC, comparing variation in different parts of selected sea vegetables species. Thallus refers to the algal body which is not differentiated in stem, leaves and roots like terrestrial plants. The current study focused on comparing blade and stipes of the brown sea vegetables, sugar kelp and winged kelp, because they are already being sold as distinct products by some producers in the northeast. For sugar kelp, the fresh and fresh frozen stipes contained 2.6 and 2.1 times more phenolics, respectively, compared to the blades (Fig 4.3). On the contrary, lower phenolic content in stipes compared to blade was reported by Connan and others (2006) in *Laminaria hyperborea* and *L. digita*, both belonging to the same genus as sugar kelp. Fresh winged kelp stipes were about the same in comparison to the blades whereas the fresh frozen stipes had lower phenolic content than the blades of the same treatment (Fig 4.4). Schmid and Stengel (2015) reported concentrations of pigments

chlorophyll *a*, chlorophyll *c*, fucoxanthin and ß-carotene to be significantly ($p<0.01$) lower in stipes compared to basal and tip parts of winged kelp blades. The same authors also reported no significant variability in pigment levels in different plant tissue for sugar kelp but mentioned this species as having a lower concentration of pigments than winged kelp.

4.3.2. DPPH Radical Scavenging Activity

DPPH results are reported as EC_{50} (mg/mL), which is the concentration of dried seaweed sample in the extraction solvent needed to inhibit 50% of the DPPH free radicals. The lower the EC_{50} of the sample, the higher its antioxidant capacity. The effects of blanching were quite evident since the EC_{50} levels were significantly ($p<0.05$) higher in the blanched samples compared to the fresh (Fig 4.6-4.9). The EC_{50} ranged from 0.9 to 26.2 mg/mL in fresh and fresh frozen treatments and from 1.7 to 133.7 mg/mL in blanched and blanched frozen treatments. Specifically, for *Gracilaria*, the EC_{50} of blanched treatments was significantly ($p<0.0001$) higher than for fresh or fresh frozen treatments, indicating lower antioxidant capacity due to blanching (Fig 4.6). A similar trend was observed in dulse, where blanching increased the EC_{50} in fresh and frozen samples significantly ($p=0.0001$) compared to non-blanched samples (Fig 4.7), resulting in approximately 75% loss of the antioxidant capacity (Fig 4.7). Although the fresh sugar kelp blades had significantly lower EC_{50} than blanched and blanched frozen, the fresh frozen treatment was not found to be statistically different from them (Fig 4.8). For sugar kelp stipes, blanching significantly decreased DPPH antioxidant capacity by 50% compared to the fresh and fresh frozen treatments (Fig 4.8). In contrast to these negative effects of blanching, Rajauria and others (2010) reported a lower EC_{50} for hydrothermally

processed (95 ºC for 15 min) fresh sugar kelp, indicating an increase in antioxidant capacity with heat treatment. However, Gupta and others (2011) reported reduction of TPC and an increase in EC_{50} of oven dried (varying temperatures) *H. elongata*, compared to the fresh samples, indicating that heat contributed to a reduction in antioxidant activity of the sea vegetables. In the current study, both the kelps had higher antioxidant capacity, with the winged kelp having an EC_{50} approximately 20-40 times lower than the red sea vegetables (Fig 4.9).

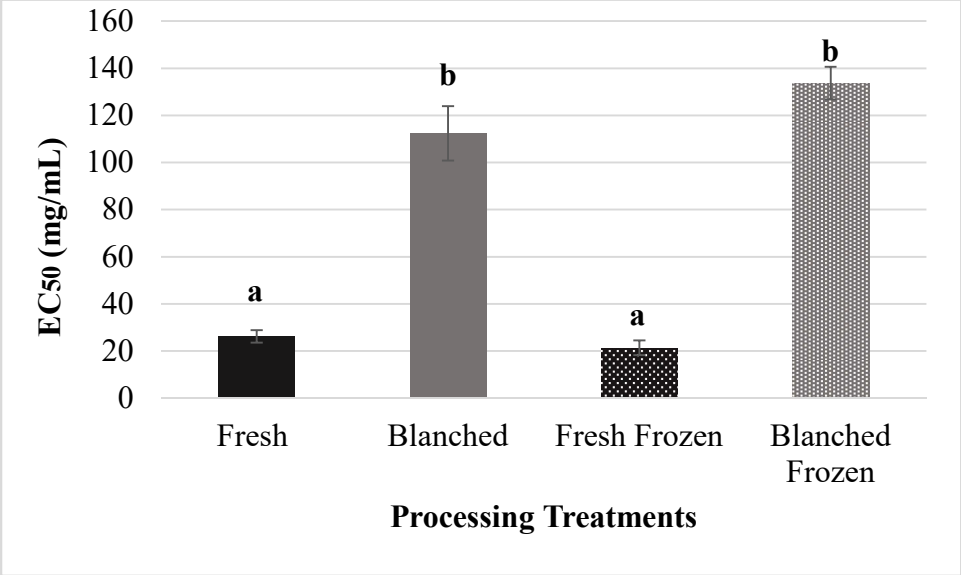
Some differences in fresh versus fresh frozen treatments were expected since any native enzymes present, such as polyphenol oxidase, catalase and lipoxygenase (Vámos-Vigyázó 1981, Nakano and others 1995, Baysal and Demirdoven 2007), commonly found in vegetables, were not blanched and may have retained some activity during frozen storage. However, no significant effects of freezing and frozen storage (one month) on DPPH antioxidant capacity were observed in the species under investigation with the exception of winged kelp. In winged kelp blades, the EC_{50} for blanched frozen samples was significantly ($p=0.0001$) higher than blanched samples (Fig 4.9), indicating a negative effect of blanching combined with frozen storage. Prior research on unblanched spinach and peas found consistent levels of antioxidant activity during frozen storage at -20 ºC for up to 3 weeks (Hunter and Fletcher 2002) and as long as 8 months in several vegetables including spinach and lettuce (Antonia Murcia and others 2009). Most vegetables are targeted to be frozen for up to 6 months to a year, however, in the current study only effects of immediate freezing were determined.

The effects of blanching on antioxidant activity were more pronounced in red sea vegetables, compared to brown. One possible explanation could be that levels of non-

water soluble pigments found in brown sea vegetables such as carotenoids and xanthophylls including abundantly present fucoxanthin (Yan and others 1999, Bocanegra and others 2009, de Quirós and others 2010, Fung and others 2013), were higher compared to the levels in red sea vegetables. Additionally, selected key pigments in red sea vegetables including phycocyanin and phycoerythrin (Bocanegra and others 2009) are present as water soluble proteins (Glazer 1994, Paull and Chen 2008), which may have been readily lost during blanching. Low radical scavenging activity has been previousely reported for dulse (Yuan and others 2005) and *Gracilaria spp*. (Zubia and others 2007), consistent with the results observed in the current study.

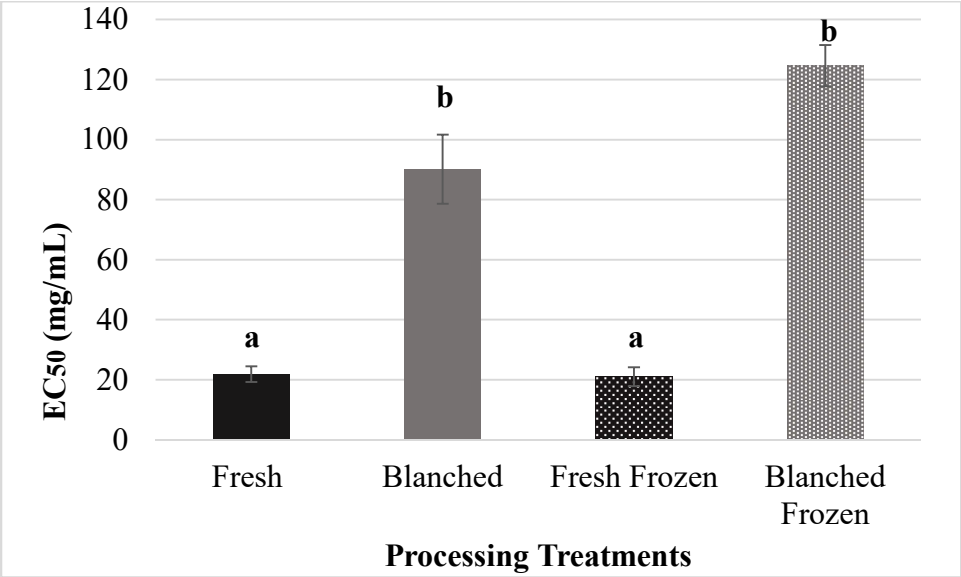
Both, sugar kelp and winged kelp stipes showed the lowest loss of DPPH scavenging activity as a result of blanching compared to all other species and product forms. This could be due to the fact that the stipes are narrower with less surface area in comparison to the flatter blades, reducing the loss during blanching of compounds that contribute to antioxidant capacity. It is interesting to note that even though both kelp species were harvested only one week apart, winged kelp showed higher radical scavenging activity compared to sugar kelp (Fig 4.8-4.9), indicating that genetic variation among kelp species plays an important role in their antioxidant activity.

Figure 4.6. DPPH radical scavenging of minimally processed *Gracilaria*



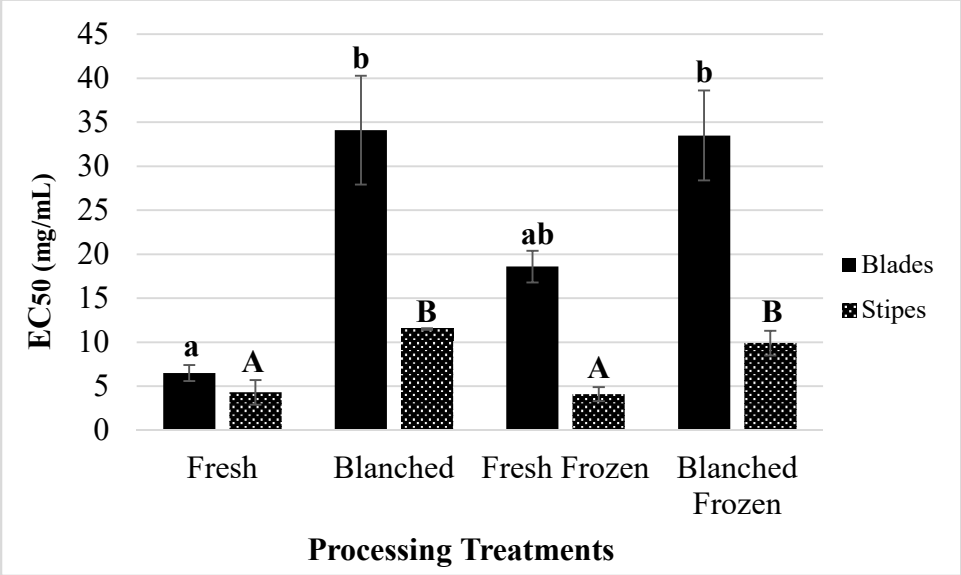
Each value is the mean ± standard deviation, (n=3). Treatments not sharing a letter are significantly ($p<0.05$) different based on one-way ANOVA followed by Tukey's HSD post-hoc.

Figure 4.7. DPPH radical scavenging of minimally processed dulse



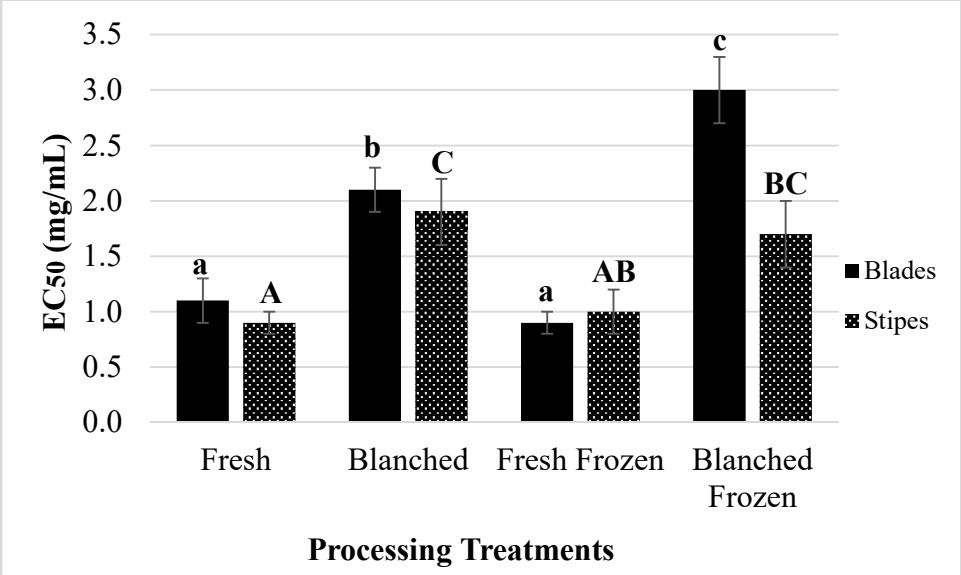
Each value is the mean ± standard deviation, (n=3). Treatments not sharing a letter are significantly ($p<0.05$) different based on one-way ANOVA followed by Tukey's HSD post-hoc.

Figure 4.8. DPPH radical scavenging of minimally processed sugar kelp



Each value is the mean ± standard deviation, (n=3). Treatments not sharing a lowercase (blades) or uppercase (stipes) letter are significantly ($p<0.05$) different based on one-way ANOVA followed by Tukey's HSD post-hoc.

Figure 4.9. DPPH radical scavenging of minimally processed winged kelp



Each value is the mean ± standard deviation, (n=3). Treatments not sharing a lowercase (blades) or uppercase (stipes) letter are significantly ($p<0.05$) different based on one-way ANOVA followed by Tukey's HSD post-hoc for blades and Student's t-test for stipes.

4.3.3. Ferric Reducing Antioxidant Power (FRAP) Assay

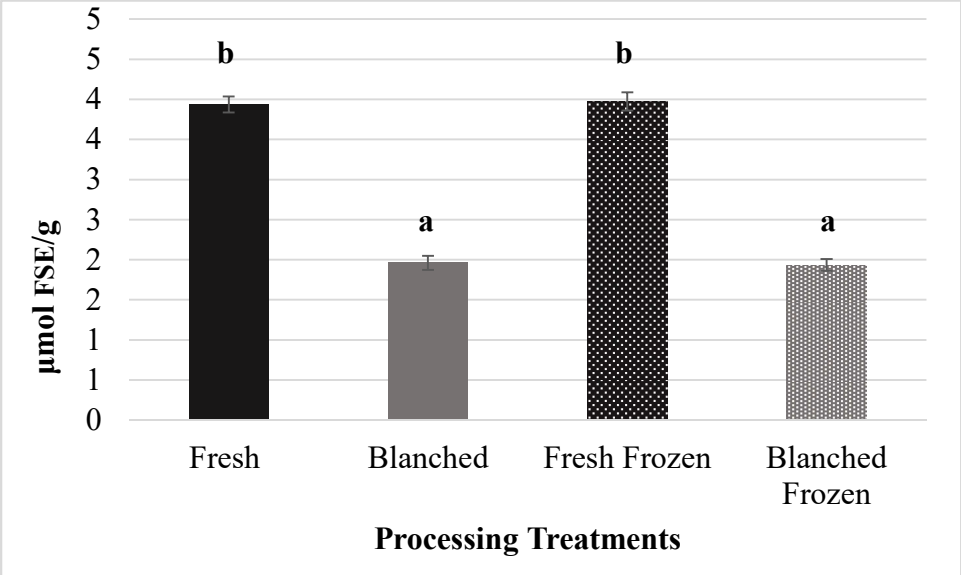
The FRAP assay is based on a single electron transfer mechanism, and assesses the ability of antioxidants in the sample to reduce ferric ion to ferrous ion (Benzie and Strain 1996, Gülçin 2014). The underlying mechanism for FRAP is not different from DPPH, as both work as electron donors. However, FRAP only uses a single electron transfer (SET) mechanism whereas DPPH uses SET and hydrogen atom transfer (HAT) mechanism to some extent (Prior and others 2005). It was important to perform both the assays to characterize the extent of both mechanisms while looking at antioxidant capacity of the seaweed samples.

Overall, the FRAP values ranged from 3.9-41.0 µmol $FeSO_4.7H_2O$ equivalents (FSE) per g dried sample for fresh samples versus merely 1.9-17.0 µmol FSE/g for blanched samples. Significant ($p<0.05$) effects of blanching were observed in FRAP values with decreased values in blanched samples compared to fresh (Fig 4.10-4.13) for all species except for dulse. In *Gracilaria*, blanching resulted in cutting the FRAP values in half, from 3.9 µmol FSE/g for fresh and fresh frozen sample to 1.8 µmol FSE/g for blanched treatments. The same change was observed in TPC of *Gracilaria* samples. For both kelps, fresh and fresh frozen blades and stipes were significantly higher in FRAP when compared to blanched and blanched frozen samples (Fig 4.12 and Fig 4.13), indicating loss of compounds with reducing power due to blanching. For dulse, only blanching in addition to frozen storage led to significant decrease in FRAP (Fig 4.11). Frozen and blanched frozen storage of winged kelp stipes resulted in significantly ($p<0.0001$) lower FRAP values in comparison to the fresh samples (Fig 4.13). However,

in all other species and product forms freezing at -20 ºC for one month did not affect the FRAP value significantly.

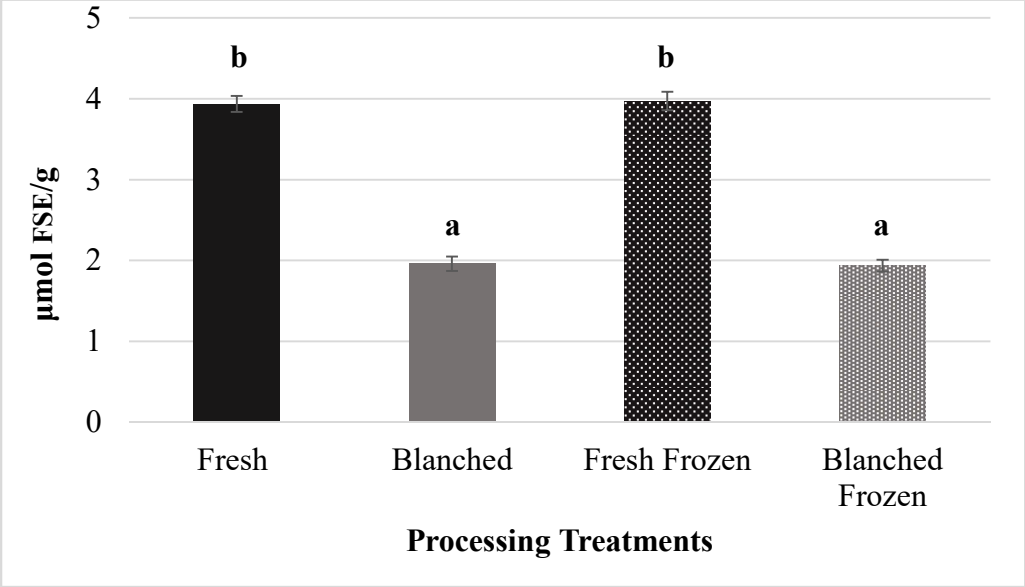
The highest FRAP value measured for red sea vegetables was 4.4 µmol FSE/g and for brown it was 41.0 µmol FSE/g. These differences indicate that the kelps evaluated in this study had higher ability to reduce the ferric ions to ferrous compared to red sea vegetables. Ferraces-Casais and others (2012) reported FRAP of fresh *Laminaria spp.* to be 6.90 µmol Trolox/g sample, which is much lower than values obtained for both the kelps in the current study. However, direct comparisons cannot be made due to different standards used in the two studies. The winged kelp samples showed higher FRAP compared to sugar kelp samples. In addition to genetic variation, the presence of a tough midrib in the winged kelp blades may have protected them against antioxidants loss during blanching.

Figure 4.10. FRAP of minimally processed *Gracilaria*



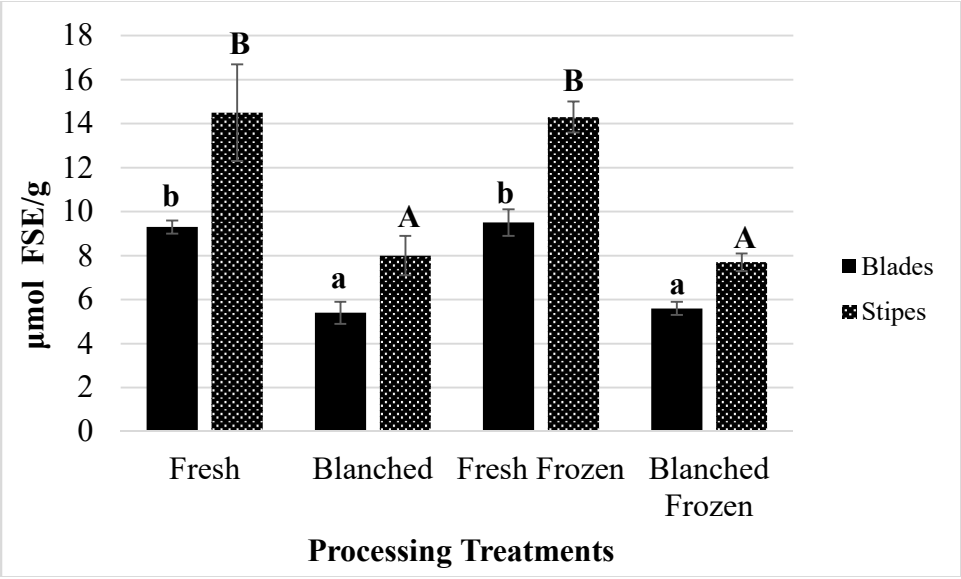
Each value is the mean ± standard deviation, (n=3). Treatments not sharing a letter are significantly ($p<0.05$) different based on one-way ANOVA followed by Tukey's HSD post-hoc. FSE = ferrous sulfate heptahydrate equivalents.

Figure 4.11. FRAP of minimally processed dulse



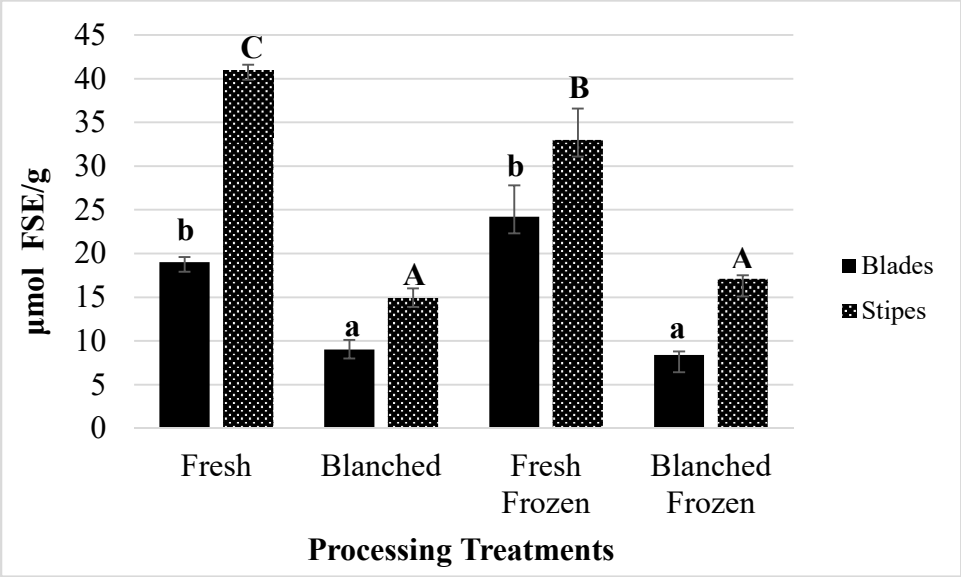
Each value is the mean ± standard deviation, (n=3). Treatments not sharing a letter are significantly ($p<0.05$) different based on one-way ANOVA followed by Tukey's HSD post-hoc. FSE = ferrous sulfate heptahydrate equivalents.

Figure 4.12. FRAP of minimally processed sugar kelp



Each value is the mean ± standard deviation, (n=3). Treatments not sharing a lowercase (blades) or uppercase (stipes) letter are significantly ($p<0.05$) different based on one-way ANOVA followed by Tukey's HSD post-hoc. FSE = ferrous sulfate heptahydrate equivalents.

Figure 4.13. FRAP of minimally processed winged kelp



Each value is the mean ± standard deviation, (n=3). Treatments not sharing a lowercase (blades) or uppercase (stipes) letter are significantly ($p<0.05$) different based on one-way ANOVA followed by Tukey's HSD post-hoc FSE = ferrous sulfate heptahydrate equivalents.

4.3.4. Correlations among TPC, DPPH and FRAP

In sea vegetables, high antioxidant activity has often been attributed to the presence of abundant phenolics (Chew and others 2008, Wijesekara and others 2011, Fernandes de Oliveira and others 2012). Their ability to play multiple roles as reducing agents, free radical scavengers, hydrogen donors and metal chelators adds to their considerable antioxidant capacity (Jiménez-Escrig and others 2001, Wang and others 2009). Correlations between TPC and the antioxidant assays were investigated for each species to determine the strength and direction of their relationship. Table 4.4 provides the Pearson's r values ($p<0.05$) for each species, treatment, and product form. For *Gracilaria*, TPC and FRAP showed a strong positive correlation (0.9676) whereas TPC and DPPH showed a strong negative correlation (-0.927), indicating that the antioxidant activity in this red sea vegetable was likely largely due to its phenolics content. Here, the

negative correlation with DPPH makes sense because the results were expressed as EC_{50}, where a lower concentration indicates higher antioxidant capacity. The FRAP and DPPH values also had a strong negative correlation (-0.96), indicating consistency among assay results. Although a strong negative correlation (-0.8447) was found between TPC and DPPH for dulse, there was a positive but moderate correlation between TPC and FRAP (0.6192). This shows that there may be other antioxidants such as selected proteins or small polysaccharides that contributed to their reducing power along with polyphenols. For sugar kelp blades and stipes, strong and positive correlations (0.8525 and 0.8707, respectively) were observed between TPC and FRAP whereas strong negative correlations were observed between TPC and DPPH (-0.798 and -0.8617, respectively). Winged kelp followed a similar trend to sugar kelp, exhibiting strong positive correlation between TPC and FRAP and negative between TPC and DPPH. These results agree with previously reported strong correlations between TPC and antioxidant assays, suggesting that polyphenols are large contributors to the antioxidant capacity in sea vegetables (Gupta and Abu-Ghannam 2011, Ferraces-Casais and others 2012, Chan and others 2013).

Table 4.4. Correlations among TPC, DPPH and FRAP

Pearson's r (p<0.05)				
Gracilaria		TPC	DPPH	FRAP
	TPC	1		
	DPPH	-0.927****	1	
	FRAP	0.9676****	-0.96****	1
Dulse		TPC	DPPH	FRAP
	TPC	1		
	DPPH	-0.8447***	1	
	FRAP	0.6192*	-0.7451**	1
Sugar kelp		TPC	DPPH	FRAP
Blades	TPC	1		
	DPPH	-0.798**	1	
	FRAP	0.8525***	-0.8498***	1
		TPC	DPPH	FRAP
Stipes	TPC	1		
	DPPH	-0.8617***	1	
	FRAP	0.8707***	-0.8982****	1
Winged kelp		TPC	DPPH	FRAP
Blades	TPC	1		
	DPPH	-0.8576***	1	
	FRAP	0.9819****	-0.8234***	1
		TPC	DPPH	FRAP
Stipes	TPC	1		
	DPPH	-0.7201**	1	
	FRAP	0.9639****	-0.7422**	1

*=p<0.05, **=p<0.01, ***=p<0.001, ****=p<0.0001

4.4. Study Limitations

This study successfully demonstrated the effects of minimal processing on antioxidant capacity of four sea vegetables commonly available in the New England area, however, more research is needed to validate the inferences from this study. Moving forward, it would be worthwhile to identify and quantify the specific phenolic compounds present in these sea vegetables to better understand their antioxidant function. Also, SET-based assays, DPPH and FRAP, were chosen to assess antioxidant capacity but including assays that use HAT, including oxygen radical absorbance capacity (ORAC) or total oxidant scavenging capacity (TOSC) as their underlying mechanism would provide additional information about the antioxidant mechanisms present in sea vegetables. Furthermore, this study focused only on one blanching and one freezing parameter. Effects of different blanching and freezing parameters should be explored in future studies to determine the best conditions with respect to maintaining antioxidants present in sea vegetables. Moreover, amplified effects of blanching imply loss of water soluble antioxidants. Upcoming studies could also assess for the presence of phenolics in the blanch water post processing to verify this hypothesis. In addition to immediate effects of freezing determined in this study, effects of long term frozen storage on antioxidant capacity must also be investigated.

4.5. Conclusions

This is the first study reporting the effects of blanching and freezing on the antioxidant capacity of fresh sea vegetables. Results indicate that blanching at 80 ºC for 1 min significantly reduced the total phenolic content and antioxidant capacity of two red and two brown species of sea vegetables. However, the effects of freezing and storing the

samples at -20 C for one month were minimal. In some cases, a combined effect of blanching and freezing were observed. The TPC, FRAP and DPPH analyses indicated a higher antioxidant capacity of brown sea vegetables in comparison to red sea vegetables. Intra-thallus variation was observed in blades and stipes of both kelps under investigation, with stipes generally having a higher antioxidant capacity than blades. Correlations between assays confirmed that the polyphenols in sea vegetable samples were likely responsible for their antioxidant capacity. Overall, the results of this study provide crucial information to support the emerging sea vegetable industry in the New England area, as it explores different processes to create new sea vegetable products.

CHAPTER 5

OVERALL CONCLUSIONS AND RECOMMENDATIONS

The results from these studies demonstrated that the rate and causes of quality deterioration during refrigerated storage were species specific and varied with season, even within species. Storage temperature played a key role in quality loss of fresh sea vegetables over time. The lower storage temperature (35 ºF) extended the acceptable quality shelf life for dulse, sugar kelp and winged kelp, but not for *Gracilaria.* This was likely due to *Gracilaria* being a summer crop, and more acclimatized to warmer temperatures. Whole fronds and shredded slaw of the two kelp species had similar shelf life. Harvest season proved to affect shelf life even within the same species, with longer shelf life for sugar kelp harvested in June compared to the February harvest. One of the major findings of these shelf life studies was the considerable and accelerated drip loss in sea vegetables over time. Sea vegetable growers can use this information to develop appropriate packaging and distribution procedures. It is recommended that the cellular liquid pooled in the storage containers/bags be discarded to maintain better appearance. All the four sea vegetables were high in total minerals, and this information can be used by the producers to market their mineral-dense fresh sea vegetable products. The low lipid content of these sea vegetables could be emphasized to attract health-conscious consumers that want to focus on diets low in fat. Vitamin C content was variable with the highest content (31.5 mg/100g) found in sugar kelp.

While the shelf life studies provided a strong foundation for future quality assessments of fresh sea vegetables, studying the effects of additional variables such as different species, product forms, packing material, and storage conditions may further

extend potential benefits to the sea vegetable industry. Moreover, consumer sensory testing of fresh farm-raised sea vegetables will provide more insights about acceptability of these products. Seasonal effects on the chemical composition of fresh sea vegetables may allow farmers to tailor their products according to harvest season, if they want to maximize levels of certain nutrients.

The literature on shelf life of fresh sea vegetables is extremely scarce. As sea vegetable producers create diverse products made with fresh sea vegetables, standard methods are needed to assess their shelf life. Methods development was not the primary objective of this study, nonetheless, the selection and development of appropriate methods was crucial to evaluating shelf life, and lessons learned laid the groundwork for future research in this area. Based on a variety of analyses, it was found that loss of cellular liquid and subsequent deterioration of color and texture were the primary causes of quality loss. Hence, quantifying drip loss provided very crucial information about quality loss over time. These results should be carefully considered when packaging and distributing fresh sea vegetables. Instrumental color (L*, a*, b* values) also provided effective data. Although, textural changes in the samples were evident throughout the study, it was extremely difficult to develop methods that provided consistent and decisive data. In the current study and in another previous study by Paull and Chen (2008), the texture data were found to be highly variable. One of the key reasons for this is that sea vegetables had high variability among and between fronds/thalli. Based on the results of this study, it is recommended that methods for assessing the texture of *specific* parts of the frond or thalli be developed.

Sensory evaluation also yielded very informative data. Panelists that were recruited had some experience with sea vegetables and they were briefly trained on each of the four species assessed. Some variations in sensory scores were observed among panelists, however, repeated training could likely decrease variability. Proper sensory evaluation demands a significant time investment but the constructive data it produces can justify the time and cost involved. Aerobic plate counts provided variable results, with counts significantly increasing during storage for certain treatments and species but not for others. Soluble protein did not change over time and was not related to quality loss in this study, which is opposite to findings of Paull and Chen (2008). Without further investigation, it is difficult to make a judgement about whether soluble protein loss is an effective method to assess quality loss in fresh sea vegetables. TVBN contents were low and not related to quality loss in brown sea vegetables.

Findings of the processing study showed that blanching at 80 ºC for 1 min reduced the total phenolic content and antioxidant capacity of all four species. On the contrary, one month of frozen storage at -20 ºC resulted in minimal effects. Based on the three analyses, TPC, FRAP and DPPH, it was clear that brown sea vegetables have higher antioxidant capacity compared to red sea vegetables. Additionally, these assays also confirmed intra-thallus variation in the two brown sea vegetables, indicating that stipes had a higher antioxidant capacity than blades. In addition to these results, testing antioxidant capacity utilizing other methods including ORAC and TOSC will deepen our knowledge on the mechanisms of these antioxidants. Moreover, since it is clear that phenolic compounds contribute greatly to the antioxidant capacity of these sea vegetables, characterizing specific phenolic compounds present will provide further

insights about the functionality of these antioxidants. As different processes including cooking, drying and canning may alter the chemical constitution of foods differently, a future focus on assessing differences in antioxidant capacity due to processing methods will help provide a more complete picture to the sea vegetable industry.

The recent rise in sea vegetable consumption in the U.S. is a sign of their wider acceptance as a part of the American diet. The small but flourishing sea vegetable aquaculture industry in New England is striving to offer their consumers a wide variety of sustainably produced, health promoting sea vegetable products. This thesis offers timely information on shelf life and processing of fresh sea vegetables, directed towards supporting the industry in achieving their goals.

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APPENDICES

Appendix A Fresh Seaweed Quality Evaluation- Dulse

Please rate the seaweed samples using the line scales provided. Make a vertical mark which represents how you perceive the intensity of each of the attributes below. To rate textural quality, pick up and handle one of the whole fronds or slaw. Please describe the reasons for your attribute ratings in the comment section below.

COLOR

l----------l
faded plum red dark plum red

AROMA
l----------l
unpleasant pleasant

SHEEN

l----------l
dull glossy

TEXTURE

l----------l
fragile strong

OVERALL QUALITY

l----------l
complete loss fresh
freshness

COMMENTS

Appendix B Fresh Seaweed Quality Evaluation- Gracilaria

Please rate the seaweed samples using the line scales provided. Make a vertical mark which represents how you perceive the intensity of each of the attributes below. To rate textural quality, pick up and handle the sample. Please describe the reasons for your attribute ratings in the comment section below.

COLOR

l----------l
faded brown-red dark brown-red

AROMA
l----------l
unpleasant pleasant

TEXTURE

l----------l
limp firm

OVERALL QUALITY

l----------l
complete loss fresh
freshness

COMMENTS

_____

_____

_____

Appendix C Consent Form – Shelf life

Dear Sensory Panelist,

You are invited to take part in a study called **"Shelf life evaluation of fresh seaweed."** This research will be conducted by Graduate Student Dhriti Nayyar and her advisor Denise Skonberg in order to complete her MS thesis project. The purpose of this study is to gather information about the length of time different species of seaweeds (or sea vegetables) can be held in refrigerated storage.

What Will You Be Asked to Do?
You will be asked to rate the quality of different species of seaweeds every few days for up to 14 days of refrigerated storage. On each test day, you will be presented with a paper ballot that will ask you to rate the color, sheen, texture, and overall quality of different seaweed samples. On each day the test should take no more than 15 minutes.

Risks
The risks that you may encounter in this study are minimal and no greater than those encountered during the handling of any fresh vegetable, including the possible exposure to an unpleasant odor at the end of product shelf life.

Benefits
There are no direct benefits to participants, but results of this study will be used to develop new products to help the seaweed industry in Maine.

Confidentiality
No personal or identifiable data will be collected. All data will be anonymous and deleted after the study is completed or within 2 years, whichever comes first.

Participation Information
Participation is completely voluntary; you may choose not to participate in the study at any time. You may skip any quality attribute that you do not wish to rate but incomplete questionnaires will not help us meet our research objectives.

Compensation
You will receive a small snack such as fruit, candy, or cookie each test day as compensation for your assistance.

If you have any questions or concerns please contact:

- Dhriti Nayyar at 315-447-3914 or Dhriti.Nayyar@umit.maine.edu
- Dr. Denise Skonberg at 581-1639 or Denise.Skonberg@umit.maine.edu

If you have questions pertaining to your rights as a research participant please contact:
Ms. Gayle Jones, Assistant to the Protection of Human Subjects Review Board, at 581-1498 or Gayle. Jones@umit.maine.edu

Your participation in this study indicates that you have read and understood the above document, and have agreed to participate in this study.

Appendix D Fresh Seaweed Quality Evaluation- SK WF

Please rate the seaweed samples using the line scales provided. Make a vertical mark which represents how you perceive the intensity of each of the attributes below. To rate textural quality, pick up and handle one of the whole fronds or slaw. Please describe the reasons for your attribute ratings in the comment section below.

COLOR

l----------l
faded brown-green dark brown-green

AROMA
l----------l
unpleasant pleasant

SHEEN

l----------l
dull glossy

TEXTURE

l----------l
fragile strong

OVERALL QUALITY

l----------l
complete loss fresh
freshness

COMMENTS

Appendix E Fresh Seaweed Quality Evaluation- SK SS

Please rate the seaweed samples using the line scales provided. Make a vertical mark which represents how you perceive the intensity of each of the attributes below. To rate textural quality, pick up and handle one of the whole fronds or slaw. Please describe the reasons for your attribute ratings in the comment section below.

COLOR

l----------l
faded brown-green dark brown-green

AROMA
l----------l
unpleasant pleasant

SHEEN

l----------l
dull glossy

TEXTURE

l----------l
fragile strong

OVERALL QUALITY

l----------l
complete loss fresh
freshness

COMMENTS

Appendix F Fresh Seaweed Quality Evaluation- Al WF

Please rate the seaweed samples using the line scales provided. Make a vertical mark which represents how you perceive the intensity of each of the attributes below. To rate textural quality, pick up and handle one of the whole fronds. Please describe the reasons for your attribute ratings in the comment section below.

COLOR

l----------l
faded brown-green dark brown-green

AROMA
l----------l
unpleasant pleasant

MIDRIB TEXTURE

l----------l
limp crisp

BLADE TEXTURE

l----------l
fragile strong

OVERALL QUALITY

l----------l
complete loss fresh
freshness

COMMENTS

Appendix G Fresh Seaweed Quality Evaluation- Al SS

Please rate the seaweed samples using the line scales provided. Make a vertical mark which represents how you perceive the intensity of each of the attributes below. To rate textural quality, pick up and handle the slaw. Please describe the reasons for your attribute ratings in the comment section below.

COLOR

l----------l
faded brown-green dark brown-green

AROMA
l----------l
unpleasant pleasant

TEXTURE

l----------l
mushy firm

OVERALL QUALITY

l----------l
complete loss fresh
freshness

COMMENTS

Appendix H Consent Form - Seaweed Salad

Dear Sensory Panelist,

You are invited to take part in a study called **"Bioactive compounds in farm raised sea vegetables."** Graduate Student Dhriti Nayyar and her advisor Denise Skonberg, from the School of Food and Agriculture at the University of Maine, will conduct this research in order to complete her MS thesis project. The overall purpose of this study is to gather information about effects of processing on antioxidant capacity of sea vegetables. You must be 18 years or older to take part in this study. Please do not participate if you are allergic to carrots, ginger, lemon, rice vinegar, brown sugar, sesame oil, sesame seeds and soy sauce.

What Will You Be Asked to Do?
If you decide to be a part of this study, you will be served three samples. For the triangle test, you will be asked to taste the three samples and pick out the different one. Following that, you will be requested to taste two samples and answer some questions about your preference. Both the tests will be conducted using paper ballots.

Risks
If you don't like sea vegetables or Asian salad dressing please do not take part in this study. The risks that you may encounter in this study are minimal and no greater than those encountered during normal eating. The test may take up to 30 minutes of your time.

Benefits
You may enjoy eating the salad. You evaluations may help seaweed industry in Maine.

Confidentiality
No personal or identifiable data will be collected. All data will be anonymous and deleted after the study is completed or within 2 years, whichever comes first.

Participation Information
Participation is completely voluntary; you may choose not to participate in the study at any time.

Compensation
Upon completion of the study, you will receive $4. No compensation will be provided if you decide to end the study without answering all of the questions.

If you have any questions or concerns please contact:

- Dhriti Nayyar at 315-447-3914 or Dhriti.Nayyar@umit.maine.edu
- Dr. Denise Skonberg at 581-1639 or Denise.Skonberg@umit.maine.edu

If you have questions pertaining to your rights as a research participant please contact:
Ms. Gayle Jones, Assistant to the Protection of Human Subjects Review Board, at 581-1498 or Gayle. Jones@umit.maine.edu

Your participation in this study indicates that you have read and understood the above document, and have agreed to participate in this study.

Appendix I Triangle Test

Taster no. _____ Date: _____

Instructions
Taste the samples from left to right. Two samples are identical; one is different.
Select the **odd/different** sample and indicate by placing an X next to the code of the odd sample. Please take a sip of water between each sample.

Samples on Tray	Indicate odd sample	Remarks
_____	☐	_____
_____	☐	_____
_____	☐	_____

If you wish to comment on the reasons for your choice or if you wish to comment on the product characteristics, please do so under Remarks.

Please proceed to take the preference test on the next page.

Appendix J Preference Test

Taster no. _____

Instructions
Fill in the codes for both samples remaining on your tray: 1- _____and 2- _____. Please taste both the samples and answer the questions below. Please take a sip of water between the tests.

1- Which sample do you prefer based on texture?

2- Which sample do you prefer based on color?

3- Which sample do you prefer overall?

Please comment on the reasons for your choice:

_____

_____

_____

_____

_____

Thank you for participating in this study.

BIOGRAPHY OF THE AUTHOR

Dhriti Nayyar was born in Mumbai, India on September 27, 1990. She was raised in Mumbai, India and graduated from Thakur College of Science and Commerce in 2006. She moved to the U.S. to pursue her higher education in 2008 and attended the North Carolina State University graduating in 2012 with a Bachelor's degree in Biological Science minoring in Genetics. She moved to Maine in 2014 and entered the Food Science and Human Nutrition graduate program at The University of Maine. After receiving her degree, Dhriti will be continuing in the same program to pursue her PhD. Dhriti is a candidate for the Master of Science degree in Food Science and Human Nutrition from The University of Maine in August 2016.