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**SUSTAINABLE POSTHARVEST PROCESSING AND VALUE-ADDITION OF
AQUACULTURED SEAWEED**

By

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A DISSERTATION

Submitted in Partial Fulfillment of the
Requirements for the Degree of
Doctor of Philosophy
(in Food and Nutrition Sciences)

The Graduate School

The University of Maine

May 2022

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ADDITION OF AQUACULTURED SEAWEED**

By Samuel Akomea-Frempong

Dissertation Advisors: Dr. Denise Skonberg and Dr. Jennifer Perry

An Abstract of the Dissertation Presented
in Partial Fulfillment of the Requirements for the
Degree of Doctor of Philosophy
(in Food and Nutrition Sciences)
May 2022

The annual growth rate of harvested edible seaweed in the United States' developing seaweed aquaculture sector leaped from 8% in 2014 to a predicted 18 – 25% from 2019 – 2025 due to increased demand. For continuous growth of the edible seaweed market, addressing challenges in food safety, perishability, processing, and product development are vital. The specific objectives of this research were to: 1) evaluate the effect of pre-freezing blanching procedures on the qualities of frozen sugar kelp, 2) evaluate the impact of blanching, freezing and fermentation on kelp quality, 3) determine the effect of rehydration temperatures on kelp quality, and 4) evaluate the survival of four pathogens inoculated on kelp stored at different temperatures.

For objective one, whole blade and shredded sugar kelp were subjected to different blanching methods, temperatures, and times, prior to one-year frozen storage. Blanching resulted in relatively higher quality frozen product than unblanched frozen

kelp. Vacuum-packed blanching at higher temperature for longer time resulted in good kelp quality for at least six months of frozen storage.

In objective two, blanching and freezing positively impacted kelp quality and consumer acceptability of kelp salad. Fermenting kelp to produce sauerkraut showed promise for new product development, and freezing prior to fermentation did not impact the overall liking scores of kelp sauerkraut. Results confirm that frozen storage is an acceptable practice prior to further value addition of kelp.

Dried kelp was rehydrated at three different water temperatures. Rehydration time decreased as initial water temperature was increased. Most kelp qualities were not notably different among rehydration treatments. However, rehydrated kelp was greener and less chewy than raw kelp, which may positively affect its consumer acceptability.

In the last study, all four pathogens survived storage regardless of the temperature. Survival for all species was greatest at $22 > 10 > 4$ °C storage. Results confirm the need for strict adherence to temperature control, and adoption of supplemental measures to enhance product safety.

These studies provide valuable information for extending the shelf-life of sugar kelp and producing high quality products, which are vital to the growing seaweed industry and for consumers of seaweed products.

DEDICATION

I dedicate this dissertation to my family and friends, who have been my source of inspiration and provide me with unconditional support.

ACKNOWLEDGEMENTS

I would like to thank both Dr. Denise Skonberg and Dr. Jennifer Perry for giving me this opportunity to pursue a doctoral degree at the University of Maine and for their invaluable mentorship, constant guidance, assistance, motivation, enthusiasm, and patience throughout this project. They believed in me and gave me endless support. I am grateful to my advisory committee members Dr. Mary Camire, Dr. Charles Yarish, and Dr. Jason Bolton, for their insightful guidance, moral support, and commitment towards my research. I would also like to thank the National Oceanic and Atmospheric Administration (NOAA) and the USDA National Institute of Food and Agriculture for financial support to start this work. Thanks to Seth Barker and Peter Fisher of Maine Fresh Sea Farms, and Springtide Seaweed for harvesting and donating fresh sugar kelp throughout this research work. I also appreciate my lab mates including Dhriti, Bouhee, Sami, Juliana, Holly, Ella, Kilee and Alison for helping and generating experimental data for this project even before I began.

I truly appreciate the collaborative efforts, attitude and optimism of the Seafood lab and Food Microbiology lab members (Adwoa, Richa, Adoum, Caitlin, Sara, Selena, Maria, Abigail, Matt and Dhafer). Mr. Emmanuel Gyaase and family, Michelle Oppong Siaw, Gertrude Arthur, Emmanuel Nimo, Seth, Bright, Henry, Oluwafemi, Esther, Adwoa and her husband, Kwaku, thank you for being my family away from home. To Richa, thank you for being there all the time and the fun trips for seaweed. I would also like to thank the Advanced Structures and Composites Center, University of Maine for giving access to the convective dryer and Adam Kuykendall of the University of Maine for taking the pictures of the seaweed salad.

My thanks and appreciation also go to Dr. Balunkeswar Nayak for his guidance and Kathy Davis-Dentici for her lab assistance in the microbiology lab, for training me on texture analyzer and colorimeter devices and her selfless support throughout my stay at the University of Maine.

Finally, to my parents, siblings, and friends for their love, prayers, sacrifices, patience, and support during my academic journey. I could not be where I am today without all of you.

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CHAPTER 1

INTRODUCTION

Individual choices regarding food selection and the amount of each food to consume are influenced by cultural, biological and economic factors including food availability, and sensory characteristics (Myers 2015). They are also influenced by associating particular sensory cues to rewarding postingestive consequences (Sclafani and Ackroff 2012), value, ease or difficulty of preparation, and the availability of other preparation tools (Smith 2006). There is increasing interest in the consumption of minimally processed foods worldwide, and the consumption of minimally processed foods may be associated with health benefits such as lower incidence of excess weight in adolescents as reported in a study conducted in Brazil (De Melo et al. 2017). The food consumption trends focusing on health and wellness are influencing growth in the global food industry (Sloan 2020a). In the U.S., healthfulness has a significant impact on food purchase for nearly two-thirds of adults (Sloan 2020a), and about a quarter of U.S. adults who shop for food, purchase fresh or raw foods at specialty grocers (Sloan 2020b). Interest in plant-based eating, veganism and vegetarianism has increased on a global scale (Fuentes and Fuentes 2021). Overall sales of plant-based food grew by 27% in the U.S. in 2020 (PBFA 2020), including the purchase of alternative meats and snacks made with grains, vegetables and seaweeds from food retailers. In addition, plant-based and plant-like foods such as lentils and seaweeds, respectively, are increasingly being utilized by food manufacturers to meet consumer demands for foods rich in nutrients.

There are several factors including various end-product innovations (Piconi et al. 2020), that contribute to the high demand for plant-like products such as seaweed. Apart

from the food industry, the pharmaceutical and other industries are also harnessing the potential of seaweed to produce medicines, animal and aquafeeds, phycocolloids and fuel among others, which are discussed later in this chapter (subheading 1.4., Seaweed industry).

1.1 Seaweed

Seaweeds are defined as large photosynthetic marine species of remarkable diversity, also known as marine macroalgae (Small 2018). Seaweeds are part of the several algal phyla in the Domain Eukarya and these algal phyla are classified within eukaryotic supergroups (Graham et al. 2016). Seaweeds are classified into three groups, namely Chlorophyta (Green), Rhodophyta (Red) and Phaeophyta (Brown), based on their coloration that is derived from the predominant pigment in the species, which aids in photosynthesis. The brown algae is part of the stramenopiles supergroup whilst the red and green algae are classified into the supergroup labeled as plants and algal relatives (Graham et al. 2016). Botanically, seaweeds do not have distinct leaves, stems or roots; nor do they flower, or produce fruit or seeds (Graham et al. 2016). According to Hurd et al. (2014), seaweeds comprise leaf-like fronds, stem-like thalli and specialized tissues termed “holdfast.” Holdfast tissues provide anchorage and may sometimes serve in nutrient uptake. Seaweeds derive their nourishment from the direct contact of their cells with the surrounding water.

Seaweeds are found in nearshore coastal areas, specifically salt water environments around the world. Most seaweeds are attached to rocks and other hard substrates including being suspended on lines or ropes, rafts, or nets for farmed seaweeds

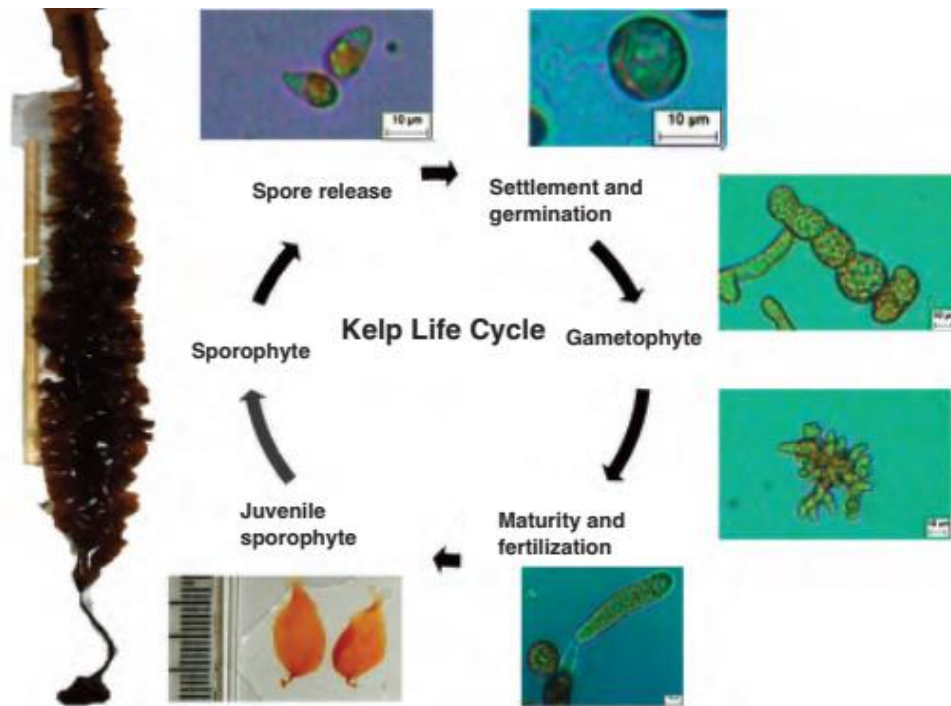
(Figure 1.1). However, some seaweed species, such as sea lettuce (*Ulva* spp.) and *Sargassum* spp., can float freely in the oceanic environments.



Figure 1.1: Sugar kelp farming in Maine, U.S., line raised for harvest

The basic life cycle pattern of seaweed alters between haploid (n) gametophytes and the diploid (2n) sporophytes (Hurd et al. 2014; Graham et al. 2016). A description of seaweed life cycles will be limited to the brown algal genus *Saccharina* since it is the genus focused on in this study. It consists of an alternation of generations between a microscopic gametophytic phase and a very large macroscopic sporophytic phase. In details, the thallus of the sexually mature *Saccharina* spp. forms sorus tissue that contains sporangia that produce meiospores, which develop into male and female microscopic gametophytes. The female gametophyte forms an egg in an oogonium and the egg is retained on the gametophyte, while the male gametophyte form antheridium, containing

motile sperm that are released into the water. The sperm fertilizes the egg, and a new sporophyte overgrows the female gametophyte as shown in Figure 1.2 below (Redmond et al. 2014).



Source: (Redmond et al. 2014)

Figure 1.2: Life cycle of *Saccharina latissima*

In aquaculture, seaweeds are typically cultivated in nutrient bioextraction or integrated multi-trophic aquaculture (IMTA) systems, whether land-based, coastal or offshore (Redmond et al. 2014). The IMTA concept is an ecologically-based model involving fed organisms (finfish and shrimp) that couples an inorganic bioextractive organism (seaweed) with an organic bioextractive organism (shellfish) to extract their nutrition from the effluents of fed organisms, in order to produce a more sustainable, cleaner, and diversified aquaculture system. Nutrient bioextraction on the other hand only has the extractive component, where both organic and inorganic bioextractive organisms

extract their nutrition from the water environment without fed organisms (Neori et al. 2007; Redmond et al. 2014). In both system it is necessary to have a source of young seaweed plants, either from isolates from wild population or from the nursery for cultivation.

Seaweeds are mainly farmed either using one-step (clonal) or multistep (non-clonal) farming processes depending on their taxa (Pereira and Yarish 2008; Bast 2014). The one-step process involves propagate by fragmentation, whereby seaweed fragments are tied to ropes or lines and/or nets, and are installed at the farm site. After harvesting, small fragments are allowed to remain attached to the substrate so that the thalli are regenerated in the next growth cycle. Some seaweeds including *Eucheuma*, *Kappaphycus*, and *Gracilaria* need one-step farming through vegetative propagation, whereas seaweeds including *Ulva*, *Laminaria*, *Porphyra* and *Undaria* must be propagated from spores with multistep farming processes and cannot survive if propagated vegetatively (Pereira and Yarish 2008; Bast 2014; Redmond et al. 2014). The multi-step approach involves *in-vitro* fertilization, or tip or spore isolation, and nursery rearing of young seedlings on plastic (PVC) spools and/or nets, before their installation in farm sites (Bast 2014; Redmond et al. 2014). In a variation on this method, seedlings of seaweed are attached to floating structures, such as ropes, at predetermined intervals with the extreme ends tied to a vertical longline kept in place by means of a surface buoy and a bottom weight, balanced with several intermediate buoys. This method is the most commonly used for kelp, where the seedlings start with setting of meiospores on seed strings that are later placed on long lines.

The cultivation, harvesting practices and the physiology of seaweed (macroalgae) help to differentiate them from other algae. Seaweed aquaculture is presently based in a relatively small group of about 100 taxa. Of these, five genera (*Laminaria*, *Undaria*, *Porphyra*, *Eucheuma/Kappaphycus*, and *Gracilaria*) account for about 98% of world seaweed production (Pereira and Yarish 2008). Across the divisions, three of the top seven most cultivated seaweed taxa (*Eucheuma* spp., *Kappaphycus alvarezii* and *Gracilaria* spp.) are used for hydrocolloid extraction (FAO 2014; Buschmann et al. 2017; Kim et al. 2017). Other industrial uses such as the production of gels, fertilizers and medicines emerged later although seaweeds were predominantly for food and feed initially. Other non-food production technologies utilize seaweed cultivation for habitat restoration (Suebsanguan et al. 2021), and as a method of removing heavy metals from marine environments (Luo et al. 2020). Currently, China, the largest seaweed-producing nation in the world, grows many types of seaweed (including kelp species, *Gracilaria* spp. and *Pyropia/Neopyropia* spp.) mostly for food (FAO 2014; Buschmann et al. 2017; Kim et al. 2017).

1.2 Edible seaweed

Most seaweeds are non-toxic, but some are poisonous. For example, the brown alga genus, *Desmarestia* contains sulphuric acid as a defense mechanism (Hurd et al. 2014; Graham et al. 2016). Among the non-toxic seaweeds, some species can be eaten like vegetables and have been utilized as food by East Asian populations (Japan, Korea, China) since ancient times (Wells et al. 2017). Introduction of the macrobiotic diet to Europe and U.S. from the East has contributed to the consumption of seaweeds or “sea

vegetables.” It has been suggested that the name “seaweed” has a negative impact on the consumption of these nutritious food products in the Western world (McHugh 2003). Therefore, a more positive term, “sea vegetables” may be used to describe edible seaweeds in Western cultures. There are edible species found among all the types of seaweed (red, green and brown) with some species eaten raw, if they are fresh and collected from areas of clean water. However, the majority are processed, either by cooking, toasting or drying, to improve flavor (FAO 2020).

Several edible varieties of seaweeds are known world-wide by names such as kombu (*Saccharina*, *Laminaria* spp.), aonori (*Monostroma* spp.), wakame (*Undaria pinnatifida*), winged kelp (*Alaria esculenta*) and nori (*Porphyra*, *Neopyropia*/*Pyropia* spp.) (FAO 2020). The nori sheet is commonly used to make sushi rolls (Figure 1.3). Other seaweeds such as wakame, hijiki and konbu (*Saccharina japonica*) are common in China, Japan, Korea, and the Philippines in a variety of products such as stews and salad. *Ulva* spp., dulse and winged kelp (*Undaria* and *Alaria*) are used in soups and salads or processed into dried snacks whereas some are pickled, toasted or eaten in jellies (Kiliç et al. 2013). Others are incorporated in rice, noodles, or soups for their umami flavor (Keyimu 2013) and possibly combined with bacon, chicken meat or dried mushrooms to provide synergy with the umami flavor (Mouritsen et al. 2012). In the U.S., Maine Coast Sea Vegetables, a seaweed company, and others, sell dulse and kelp commercially as salt alternatives to be used in various kinds of food preparations.



Source: Vivapura.com

Figure 1.3: A nori sheet (left) and nori sheet used in sushi rolls (right)



While some of these edible seaweeds such as arame (*Ecklonia bicyclis*), dulse (*Palmaria palmata*), and hijiki (*Sargassum fusiforme*) (Mouritsen et al. 2013) are wild harvested from the sea, others including *Kappaphycus*, *Saccharina* and *Chondrus* are mostly farm-raised in various parts of the world (FAO 2020; Augyte et al. 2021). Among all these edible seaweeds, data indicate red seaweed as the most widely cultivated (53.5%) followed by brown (46.1%) and then green seaweeds (0.4%) in about 40 countries in the world (FAO 2020; Chopin and Tacon 2021).

1.2.1 Red seaweed

Water-soluble phycobiliprotein pigments such as phycoerythrin and phycocyanin are found in the thylakoids of the cells of red seaweeds (Graham et al. 2016). These pigments are responsible for the color in red seaweeds, ranging from dark red to bright pink depending on the species (Bocanegra et al. 2009). Based on the cell biology (similarities of the plastids in their cells) and genetic analysis of red seaweeds they are supposedly related to green seaweeds. Apart from different pigmentation, the plastids of red seaweeds do not contain starch, but different branched glucans, known as floridean starch, are produced in the cytoplasm (Graham et al. 2016). Red seaweeds are diverse and

abundant in tropical and temperate marine waters, where they play important ecological roles. A typical example is the corallines, which help build and maintain coral reefs that harbor diverse organisms (Graham et al. 2016). Various species of red seaweeds include *Mastocarpus stellatus*, *Kappaphycus alvarezii* and *P. palmata*, with details of two edible red seaweed species shown in Table 1.1.

Table 1.1: Characteristics of two commercially relevant red seaweeds

Name	Description	Geographical location	Harvesting times	Uses	Picture	References
Dulse (<i>Palmaria palmata</i>)	<p>-A perennial with a morphological structure that resembles the fingers on a hand</p> <p>-Dark red or purple leathery blades with varying widths and lengths up to half a meter.</p> <p>-Lighter shade of color in dulse after harvesting due to loss of water-soluble pigments</p>	<p>Harvested mainly in:</p> <ul style="list-style-type: none"> -Ireland -Shores of the Bay of Fundy in eastern Canada -Canadian-US border between New Brunswick and Maine 	mid-May to mid-October	<ul style="list-style-type: none"> -Dried or toasted to increase palatability -Prepared salads -Dried dulse powder as additive in flour to improve nutritional content 	 <p>Source: sciencephotolibrary.com</p>	<ul style="list-style-type: none"> -McHugh 2003 -Morrissey et al. 2001 -Mouritsen et al. 2012 -Mouritsen et al. 2013
Pacific dulse (<i>Devaleraea mollis</i>)	<p>-A biennial or perennial.</p> <p>-Light yellowish pink or light red to medium red, not shiny.</p> <p>-Lacks midrib and can grow up 30 cm or more in length.</p>	<ul style="list-style-type: none"> -Semi-protected and semi-exposed shorelines of Bering Sea and Aleutian Islands in Alaska to southern California -Russia 	April and May	<ul style="list-style-type: none"> -An in situ biofilter -Feed in land-based abalone culture -Dried and sold as a cooking ingredient or nutritional supplement 	 <p>Source: centralcoastbiodiversity.org</p>	<ul style="list-style-type: none"> -Demetropoulos and Langdon 2004 -https://www.centralcoastbiodiversity.org/red-ribbon-bull-palmaria-mollis.html

1.2.2 Green seaweed

Green seaweeds are found in marine and freshwater environments unlike red and brown algae, which are restricted to marine conditions. Cell biology, biochemistry and evolution reveal that the division Chlorophyta, in which green seaweeds are found, has a similar lineage to land plants (Graham et al. 2016). The color of green seaweeds is influenced by the presence of chlorophyll *a* and *b* in the chloroplast of their cells, resulting in different shades of green (McHugh 2003), although some have red protective pigments hidden in the chlorophylls. Green seaweeds are important sources of food for aquatic animals and humans, and some representatives form significant symbiotic partnerships with some freshwater protists and invertebrates (Graham et al. 2016). Examples of green seaweed include *Codium fragile*, *Acrosiphonia coalita* and *Ulva* spp.

Sea lettuce (*Ulva* spp.)

Sea lettuce forms noticeable large, broad flat blades, which can be as long as one meter and are composed of two cell layers. The blades are ruffled, pale green, very thin and are attached to the holdfast composed of rhizoids. They normally attach their rhizoidal branches or holdfast to substrates in marine coastal waters or can be found in free-floating masses (Graham et al. 2016). Sea lettuce thrives well in seawater that has low salinity and does not do well in areas where large quantities of nutrients are present. However, it is efficient in removing ammonium from water and its lower resistance to water current makes *Ulva* spp. a suitable biofilter in fish aquaculture (Shpigel et al., 1997; Neori et al., 1998) . *Ulva* spp. are found at all levels of the intertidal zone (Figure 1.4) and are some of the most widely consumed green seaweeds in the world. In Japan,

Ulva is an ingredient for ao-nori and is used as small flakes that are sprinkled on warm rice (Mouritsen et al. 2013; FAO 2020).



Source: (Graham et al 2016)

Figure 1.4: A picture of sea lettuce (*Ulva lactuca*)

1.2.3 Brown seaweed

The color of brown seaweeds is affected by environmental factors such as temperature, light intensity, nutrients and water pH. It also depends on the species, ranging from light olive to golden brown to dark brown (Hurd et al. 2014; Graham et al. 2016). Some of these species are annual and others are perennial, living up to about 15 years. Brown seaweeds form large biomasses in intertidal and subtidal coastal regions throughout the world as a result of their size, productivity, and longevity (Graham et al. 2016). One common genus among brown seaweed is *Fucus*, which is abundant in the intertidal region of temperate rocky shores, and other examples are kelp (including species of *Saccharina*, *Macrocystis*, *Laminaria*, *Alaria* and *Undaria*), *Sargassum*, *Ectocarpus*, Dictyolaes, and Chordariales. The next section will put emphasis on kelp since it is the primary domestically grown edible seaweed variety in the U.S., with sugar

kelp (*Saccharina latissima*) and alaria (*Alaria esculenta*) contributing about 80% and ~15%, respectively, of the market share (Piconi et al. 2020).

Kelp

Kelp is a term for about 300 different species of brown algae. Kelp thrives under the surface of the water and can form enormous forests, anchored to the ocean bed and reaching as far as 50 meters up into the water (Mouritsen et al. 2013). Some edible kelp include giant kelp (*Macrocystis pyrifera*), giant bullwhip kelp (*Nereocystis luetkeana*), winged kelp (*Alaria esculenta*, *Undaria pinnatifida*), sugar kelp (*Saccharina latissima*) and skinny kelp (*Saccharina angustissima*). Kelp can be used in the singular or plural form. The type and age of kelp affects its texture, whether thin or thick, soft or tough (Carney et al. 2005; Graham et al. 2016). Kelp is a good source of vitamins and minerals, especially iodine, which is essential for thyroid health (Brown et al. 2014). Kelp also contain alginic acid, a soluble polysaccharide that has been found to aid in weight loss (Georg Jensen et al. 2013; Brown et al. 2014). Almost all kelp production formerly occurred in China (88.3%), South Korea (6.6%) and North Korea (4.4%) (FAO 2016). In Western countries, kelp species, especially *S. latissima* and *A. esculenta*, have been successfully cultivated in the United States, Iceland, Canada, Norway, Scotland, Germany and Sweden (Kim et al. 2017). According to Redmond et al. (2012) kelp species are native to New England in the U.S., and are traditionally wild harvested for food (Figure 1.5). In Maine, *S. latissima*, *S. angustissima*, *A. esculenta* and *Laminaria digitata* (horsetail kelp) are the four commercially important kelp species.



Source: Redmond et al. 2012

Figure 1.5: Sugar kelp and kelp salad (right)

Saccharina latissima grows to about 8 – 10 m in length, and occupies the lower intertidal and subtidal zones of the North Atlantic and the northeast Pacific as well as the Arctic Ocean and Baltic Sea in Europe (Egan and Yarish 1988; Bolton 2010). *S. latissima*, with regard to seaweed aquaculture in Maine, has been successfully cultivated and is the subject of many research projects to promote its production (Redmond et al. 2014; Piconi et al. 2020). *S. latissima* growth is based on temperature, light intensity and availability of nutrients such as nitrates and phosphates for absorption (Pereira and Yarish 2008; Redmond et al. 2014). Because they require little light, *S. latissima* can grow deep in the ocean (Egan and Yarish 1988). However, they undergo regulated, photoprotective responses to high levels of solar radiation that involve changes in photosynthetic efficiency (Graham et al. 2016). *S. latissima* is mainly cultivated because of its high biomass yields within a short period (Kim et al. 2015a, 2017; Augyte et al. 2017). The matured thalli are harvested from late March to early June in Maine and other sites in the northern hemisphere when the water is still cold. It is known as sugar kelp due to its sweet flavor produced by a substantial amount of the sugar alcohol mannitol found in the blades in late spring. Sugar kelp has a rich umami flavor that can be applied to various foods as a flavor enhancer (Chapman et al. 2015). The broad range of *S. latissima*

applications, include as food for humans (soups, salads), animal feeds, soil fertilizer, cosmetics and most importantly use in the alginate industry (Hardouin et al. 2014).

1.3 Nutritional benefits

The nutritive value of seaweed depends on the species and their chemical composition (Graham et al. 2016). The nutrients of seaweeds are beneficial to humans, plants and animals (Vijayakumar et al. 2019; Morais et al. 2020). Seaweed are also used as a fertilizer which is suitable for use in organic agriculture (Vijayakumar et al. 2019). They provide direct nutrition for larvae of mollusks, echinoderms, rotifers, daphnia, and crustaceans as well as some fishes, promoting growth and development from the juvenile to the adult stage (Valente et al. 2006). Seaweeds are normally mixed with feed of both monogastric and ruminants as nutraceuticals, a term that results from the combination of nutritional and pharmaceutical, used to identify food components that bring health benefits, including the prevention of some diseases (Morais et al. 2020).

Scientific research conducted on seaweed has shown the presence of many nutrients that improve human health (Holdt and Kraan 2011). Seaweed can be high in protein, at up to 47% of dry weight in red seaweed such as *Porphyra tenera* (Černá 2011; Anis et al. 2017). Protein content is highest in red, then green, then brown seaweed (Wong and Cheung 2001; Cian et al. 2014; Anis et al. 2017). Also, seaweed contains many or all of the essential amino acids (histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, valine), making it a complete source of protein (Lourenço et al. 2002; Mæhre et al. 2014; Fleurence et al. 2018). Although most seaweeds have low lipid and fatty acid content, McDermid and Stuercke (2003) reported

higher levels of lipids (~16-20% dw) in some brown seaweeds. The fatty acid profile of seaweed consists of a high polyunsaturated fatty acid (PUFA) content, which includes omega-3 and -6 PUFA (eicosapentaenoic acid “EPA” and docosahexaenoic acid “DHA”) that are lacking in land-based plants (Sánchez-Machado et al. 2004; Wells et al. 2017). The percent EPA of total fatty acids ranges from 2-14% in brown, 8-59% in red and 0.8-6% in green seaweed. DHA ranges from 0-13% in brown, 0-0.5% in red and 0-1.1% in green seaweed (Fleurence et al. 1994; Matanjun et al. 2009; Van Ginneken et al. 2011; Rodrigues et al. 2015).

Seaweeds are a rich source of structurally diverse bioactive components such as phlorotannins, sulfated polysaccharides and pigments (Sanjeeva et al. 2018). Most of these bioactive components are produced by seaweed as a protectant against abiotic and biotic stresses, such as herbivory and sea mechanical motion. The seaweed species (Holdt and Kraan 2011), reproductive status, location, depth in water, salinity, light intensity exposure, ultraviolet radiation, intensity of herbivory, and time of collection affects the amount of bioactive components in the product (Cotas et al. 2020). Phlorotannins, bromophenols, flavonoids, phenolic terpenoids and fucoxanthin have been extensively studied in brown seaweeds (Cotas et al. 2020). These compounds have been reported to possess a number of bioactivities such as radioprotection, antioxidant, antidiabetic, antimicrobial, antiobesity, and anti-inflammatory properties (Peng et al. 2011; Sanjeeva et al. 2018; Cotas et al. 2020).

The mineral content of seaweed is high, which is reflected in its high ash content (Sánchez-Machado et al. 2004). According to Makkar et al. (2016) the average mineral content in seaweed is 10-20 times higher than in land plants, and red and green seaweeds

are higher in minerals than brown seaweeds. For vitamins, a study reported water-and lipid-soluble vitamins, including B₁, and E, in higher quantities in seaweed than other vitamin-rich foods such as carrots, oranges and beef liver (Fabregas and Herrero 1990). Seaweed contains polysaccharides and one major polysaccharide (polyuronic saccharide) found in the intercellular matrix (cell walls) of brown algae as a gel containing sodium, calcium, magnesium, strontium and barium ions is alginate, making up to 14-40% of the dry mass (Draget 2009). These ions in alginate have metal chelating properties that enable them to scavenge toxic elements in the human gut and decrease cholesterol uptake when consumed (Brownlee et al. 2005). Table 1.2 summarizes the nutrient composition of various seaweeds as reported by other studies around the world.

Table 1.2: Some nutritional constituents found in seaweed

Seaweed	Brown		Green <i>Ulva</i>	Red		References
	<i>Laminaria & Saccharina</i>	<i>Fucus</i>		<i>Chondrus</i>	<i>Palmaria</i>	
Moisture (% of wet wt.)	84 -87% ^a (fronds) 73 - 90% ^b 94% ^c	68-75% ^d 70-87% ^e	78% ^b 80% ^{fg}	72% ^h 80% ⁱ	84% ^j (wild & cultivated)	^a Horn (2000); ^b Foti (2007); ^d Baardseth and Haug (1953); ⁱ Lamare and Wing (2001); ^h Larsen and Haug (1958); ^j Mishra et al. (1993)
Ash (% of dry wt.)	15 - 37% ^a 16 - 45% ^a (fronds)	19% ^b 25% ^c 30% ^d	11% ^e 13 -22% ^f 52% ^g	21% ^h	12 - 37% ⁱ 15% ^j (wild) 27% ^j (cultivated)	^a Jensen and Haug (1956); ^b Marsham et al. (2007); ^e Ortiz et al. (2006); ^h Baardseth and Haug (1953); ^j Mishra et al. (1993)
Total polysaccharides (% of dry wt.)	38% - 61% ^{abc}	62% ^b 66% ^c	15 -65% ^d 42 -46% ^e	55 -66% ^f	38-74% ^g	^a Wen et al. (2006); ^{bf} Morrissey et al. (2001); ^d Ortiz et al. (2006); ^g Heo and Jeon (2009)
Total structural & dietary fiber (% of dry wt.)	36% ^a		38% ^{ab}			^a Dawczynski et al. (2007); ^b Lahaye (1991)
Mannitol	2 - 19% ^a					^a Haug and Jensen (1954)
Laminaran	0 - 33% ^a	84% ^b				^a Haug and Jensen (1954) ^b Rioux et al. (2007)
Total protein (% of dry wt.)	3 - 14% ^a 5 -20% ^b (fronds)	1.4% ^c 5 -10% ^d	4 - 9% ^e	6 - 7% ^f 13 -18% ^g 29% ^h	8 - 35% ⁱ 12 - 22% ^j	^a Jensen and Haug (1956); ^c Rioux et al. (2007); ^e Barbarino and Lourenço (2005); ^j Smith and Young (1953)
Total fatty acids	0.3 - 1.8% ^a 0.7 - 2.9% ^b	0.5-1.5% ^c 3.1% ^c	0.3% ^d 0.6 -1% ^e 1.6% ^f	0.7% ^g 1 - 3% ^h	0.2 - 3.8% ⁱ	^a Jensen and Haug (1956); ^c Kim et al. (1996); ^d Ortiz et al. (2006); ^g Tasende (2000); ⁱ Morgan et al. (1980)
Iodine (mg 100/g of dry wt)	23 - 211 ^a 150 - 1200 ^b	73 ^a	2.0-25 ^c	1.1 ^d 20-30 ^e	10-100 ^c 15-55 ^e	^a van Netten et al. (2000); ^c Mabeau and Fleurence (1993) ^e Morrissey et al. (2001)

Adapted from Holdt and Kraan (2011).

Due to the beneficial chemical composition of seaweeds, they have been used medicinally by indigenous peoples in many parts of the world. Compounds such as terpenes (metabolites) found in seaweed are reported as potent drugs against cancer, malaria and heart diseases (Chen et al. 2018; Freile-Pelegrín and Tasdemir 2019). Brown algae has been used for treating goiter in China (Levine 2016), the jelly extract of *Chondrus crispus* was recommended against cough, diarrhea, dysentery and gastric ulcer and *Hypnea nidifica* was used in stomach ailments in the Hawaiian Islands (Anis et al. 2017). A study reported anticancer properties of ethanol extracts from *Porphyra tenera* on oral cancer cells (YD-10B). The study revealed that an exposure of YD-10B cells to the *P. tenera* extracts (50–200 µg/mL) for 24 or 48 h induced apoptosis cell death in YD-10B cells (Kim et al. 2015b; Sanjeewa et al. 2018). Moreover, polyphenolic compounds in seaweeds inhibit both α -amylase and α -glucosidase activity, potentially reducing the rates of diabetes (Brown et al. 2014). It is also interesting to note that low rates of heart diseases and obesity in Japan are often attributed to their high consumption of seafood products including seaweeds (Brown et al. 2014).

1.4 Seaweed industry

The purposes for seaweed production vary (Figure 1.6), with the current global seaweed industry focusing more on seaweed for food, feed, and food additives with medicinal products as a secondary market (Jean-Baptiste 2018).

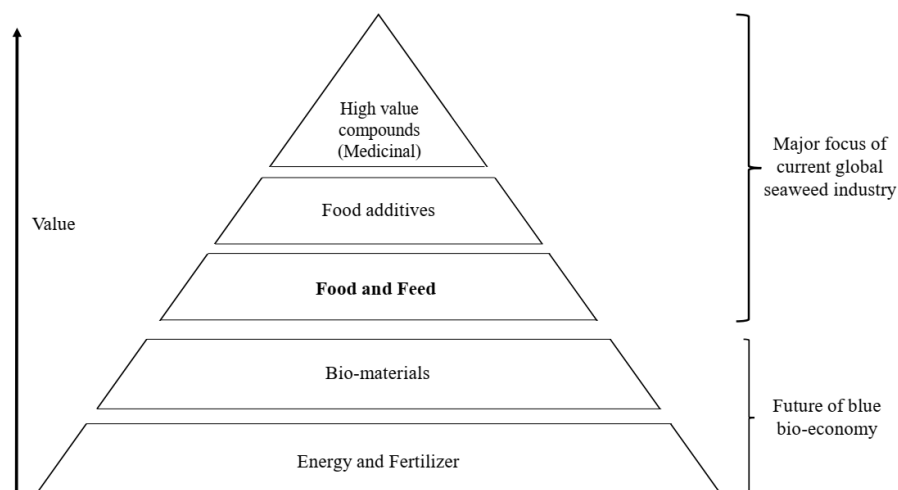


Figure 1.6: The biomass value pyramid adapted to the seaweed industry (Jean-Baptiste 2018)

Seaweed for human consumption represents an estimated 85% of total global seaweed production, including finished products, ingredients for beverages, thickening and gelling agents in food (Porse and Rudolph 2017), nutritional products, etc. For aquaculture-sourced seaweed, human food products account for more than 90% of production (Piconi et al. 2020). Seaweeds are used as food items including ready-to-eat vegetables and as an ingredients, such as seaweed extracts for hydrocolloids, by the food industry. Three types of hydrocolloids have been extracted from red and brown seaweeds, namely agar, alginate and carrageenan (Khalil et al. 2018) and these are mostly used in dairy products (Khalil et al. 2018). Agar extracted from red seaweeds is used as a gelatin substitute in vegan food products. Alginates from the brown seaweeds *Ascophyllum* and *Durvillaea* are used in making jellies (Razavi 2019). For the non-food industry, seaweeds are exploited or potentially being exploited for several reasons. In the medicinal and pharmacological industry, seaweed has been used to prevent diseases and also to protect against the most prevalent deficiency diseases such as endemic goiter and nutritional anemia from lack of iodine and vitamin B₁₂, respectively (Anis et al. 2017).

Hydrocolloids such as agarose, alginate, carrageenan, and ulvan biopolymeric gels are used in these industries too for cartilage tissue regeneration treatment and tissue engineering, as well as for wound healing and dressing (Popa et al. 2014; Venkatesan et al. 2015; Porse and Rudolph 2017). Seaweeds have great potential for cosmeceuticals. They have bioactive components such as vitamins (vitamin C, vitamin E, retinol), antioxidants and polysaccharides (carrageenan, ulvan), among many other constituents, which are used in cosmetics in the production of soaps and skin nourishing lotion as moisturizers, cleansers, antiaging and UV-protectants (Couteau and Coiffard 2016; Anis et al. 2017). They are also used in the textiles, paper and fiber industries (Oualid et al. 2020; Saleh et al. 2021), and in growth media for laboratory experiments in the field of microbiology and biotechnology. Seaweed is also used as feedstock for biofuels production including biogas, bioethanol and biodiesel (Michalak 2018), to overcome the short-comings of first and second generation of biomass from land crops, although some technical and engineering difficulties remain to be resolved (Milledge et al. 2014). There is a potential seaweed application in the agro-chemical industry as well. Some secondary metabolites (polyphenols, alkaloids, terpenes and stilbenes) and compounds such as halogenated alkanes and alkenes, sulphur-containing heterocyclic compounds (sulphated polysaccharides) and phlorotannins (Watson and Cruz-Rivera 2003) in seaweed have been found to exhibit some bacteriocidal or bacteriostatic properties. Some of these extracts from seaweed were found to be effective against pathogens such as *Vibrio* spp., *Yersinia pestis*, and *Streptococcus* spp. associated with chicken (Lubobi et al. 2016). Thus, seaweed is being considered in the poultry industry to reduce antibiotic usage where antibiotic-resistant bacteria commonly thrive. Other industries are focusing on

seaweeds for bioaccumulation to reduce heavy metals content in wastewater as well (Roleda and Hurd 2019; Kang et al. 2021).

1.5 Economic importance of seaweed

The global seaweed market is diverse and growing. Production and processing of seaweeds provide significant income and support to coastal and remote rural communities worldwide, particularly in southern Africa and Asia (Monagail et al. 2017). Reporting incomes and employment in the wild harvest seaweed industry is difficult because only a small fraction of those who work gathering seaweeds are employed in a full-time role (Monagail et al. 2017). The first and most significant direct economic benefit of gathering wild seaweed is associated with subsistence (Salo et al. 2014); as seaweed harvesting rarely accounts for the main income of the household, but rather it is an additional income for members of coastal communities who normally fish after seaweed harvesting periods (Monagail et al. 2017). The selling of locally derived products helps rural communities earn supplementary income where limited revenue sources may be available (Salo et al. 2014). Currently, a lot of companies are springing up in the production and processing of seaweed, including seaweed aquaculture operations, due to the escalating global demand for seaweeds and their products (Kiliç et al. 2013; Kim et al. 2019b).

The global annual seaweed harvest represents almost 80 billion pounds (36 million metric tonnes), with a harvest value of approximately \$11.4 billion USD across all species and end-product formats in 2020 (FAO 2020). Whereas seaweed from aquaculture accounts for almost 97% of global supply, or an estimated 77 billion pounds

(Piconi et al. 2020). There have been significant increases in seaweed production in the U.S. from 8,207 to 11,113 tons between 2009 and 2016 (FAO 2016), and about 19 million dry weight pounds in 2019 (Piconi et al. 2020). Out of this mass, Maine and Alaska are the leading domestic edible seaweed producers, accounting for more than 85% of total U.S. production (Piconi et al. 2020). The wild edible seaweed harvest was 230,445 wet pounds in Maine and valued at \$105,177. Recently, domestic edible seaweed harvest in Maine was projected to increase at an annual growth rate of 11.5% - 18.5% from 2019 to 2025. In Maine, the price of wet *Saccharina latissima* or *Alaria esculenta* was \$0.26 - \$1.00 per pound at harvest and \$0.5 - \$2.00 per pound for organic products in 2019 (Piconi et al. 2020). A lot of emphasis is now placed on minimal processing, value-addition and customer relevant products to increase prices of seaweed. While dry *S. latissima*/*A. esculenta* may cost around \$3.00 - \$10.00 per pound with its organic counterpart costing \$8.00 - \$16.00 per pound, finished processed products such as roasted seaweed snacks may be priced around \$10.00 - \$50.00+ per pound.

1.6 Harvesting and postharvesting handling

Harvest time is greatly influenced by the type of seaweed. Most seaweeds are harvested during spring to early fall each year. In wild harvest, seaweeds are either gathered from the beach using rakes, directly from their habitats by hand cutting with sickles on rocks at low tide, or with dragnets (Radulovich et al. 2015). Mechanical harvesting, by boat or trucks, has been successful in several northern Atlantic countries for decades. Regarding farmed seaweeds, harvesting can range from manually bringing in an armful on foot from intertidal off-bottom plantings to mechanized harvesting of

floating lines from large barges in deeper waters (Radulovich et al. 2015). Harvesting can be partial, where new growth of some seaweed species are cut leaving stock to regrow. The harvesting methods and intensity of exploitation affect the regenerative process of cut seaweed. Therefore, the well-being and sustainability of seaweeds are influenced by the appropriate use of the right tools (Monagail et al. 2017).

Postharvest practices are crucial for seaweed quality and shelf-life, therefore transporting seaweed to land is key and a relatively costly aspect of sea farming. These postharvest practices normally begin with cleaning of harvested products either on the water or on shore. Cleaning comprises the removal of debris, snails, bryozoans and tying strings, cutting of damaged parts and washing with seawater and/or freshwater (Radulovich et al. 2015). After cleaning, drying is the most common postharvest process, although seaweeds may also be consumed or processed fresh. Globally, most seaweeds are sundried, potentially exposing them to rain, contamination and uneven drying; with methods like forced air and heat-assisted solar dryers and ovens increasingly being used recently (Radulovich et al. 2015). Before drying, seaweeds are flattened or cut to the desired shape and some are salted to enhance preservation. However, there are other and novel methods that are being tested to preserve seaweeds as well (Radulovich et al. 2015; Maine Coast Sea Vegetables 2016; López-Pérez et al. 2020).

1.7 Processing of seaweeds

Most harvested seaweeds are processed rather than consumed fresh for several reasons. Some of these processing methods include drying (Sappati et al. 2019), refrigeration (Nayyar and Skonberg 2019), freezing (del Olmo et al. 2019), and salting

(Perry et al. 2019; López-Pérez et al. 2020). The aim of these seaweed processing methods in the food industry is mainly to extend shelf-life of the produce since seaweed has high moisture content that can facilitate the growth of spoilage microorganisms during storage.

1.7.1 Salting

Salting is one of the oldest methods of food preservation. It has been used in preserving numerous food products including fish, meat and vegetables. Salting of vegetables is common among Middle Eastern countries and traditionally is used to preserve surplus vegetables, whether fresh or semidried (Bautista-Gallego et al. 2013). Vegetables are salted either by dry salting or brine salting. In dry salting, vegetables are graded, sorted, and trimmed if they are bulky or root vegetables, before air-drying prior to salting. After sprinkling dry salts onto the surface of the vegetables at a desired ratio of salt to vegetables, kneading, mixing and squeezing are performed to facilitate the exudation of moisture. Once salted and covered tightly, products are stored for up to several months depending on the storage temperature. For brine salting, minimally processed vegetables such as eggplants, bamboo roots, and cucumber are packed in a concentrated brine as high as 20% salt for preservation (Wang 1999). Protein denaturation and precipitation can occur in vegetables during salting because of protein salting-out and high ionic forces, which have been observed in salting of fishes. However, protein precipitation and solubilization are time and NaCl-concentration dependent (Barat et al. 2002). Particular attention is needed in salting brown seaweeds (kelp) to retain the protein profile, since they have lower protein contents as compared to red and green seaweeds (Wong and Cheung 2001; Cian et al. 2014). Sugar kelp has high

levels of iodine (Wells et al. 2017), thus particular attention on salting sugar kelp is necessary as some commercially available salts contain iodine. Salting imparts a distinctive flavor to vegetables and results in low caloric values and reduction of vegetable mass for easier storage (Li and Hsieh 2004). High concentrations of salt inhibit microbial growth, while low concentrations are used in fermentation for microbial growth, which helps provide acidic pH. Feng-Di et al. (2007) researched the impact of salting with different concentrations between 0% and 12% (w/v) on Chinese cabbage over time. Mesophilic bacteria increased with time in samples without salt, while the presence of salt helped to inhibit microbial growth. Within the first 12 hours for salt concentrations below 4%, no obvious mesophilic bacteria growth was detected but population increased by two log cycles between 12 and 30 hours. However, at salt concentrations above 5%, the level of mesophilic bacteria cell counts reduced more quickly from 0-12 hours than from 12-30 hours with only 12% (w/v) salt concentration (between 5-5.5 log CFU/g) being significantly different from control samples of 0% w/v salt (between 7-7.5 log CFU/g) at 30 hours. Regarding seaweed, Perry et al. (2019) dry salted ~25 cm long sugar kelp pieces at five different salt concentrations (0, 30, 50, 180, 200 g/kg) and total bacteria and fungal counts remained low (<3.5 log CFU/g) throughout 90 days of refrigerated storage (5 °C), suggesting minimal risk of spoilage from psychrotrophic microorganisms. However, salt treatment above 50 g/kg resulted in a significantly lower concentration of calcium, magnesium and potassium as compared to unsalted kelp samples. When the highest three salting treatments were used to prepare Asian style salads for consumer acceptance testing, scores indicated that consumers liked the color and texture of the least salted product samples (50 g/kg) significantly more than

that of 180 g/kg and 200 g/kg kelp samples. Results were promising as salting could be utilized in preserving seaweed, and the salted products were slightly liked by consumers based on a hedonic acceptability test. Application of salting seaweed is used in commercially available products such as roasted seaweed snacks, however optimizing the salting process of seaweed would yield consistent desired flavor, extend the shelf-life and increase final product availability. A 2008 study reported a quick (14 days) discoloration of *Gracilaria* parts not submerged in seawater when stored at 5 °C as compared to those completely submerged in seawater (brining) (Paull and Chen 2008). It would be important to subject other seaweeds to brining and dry salting to evaluate their effect on quality and shelf-life.

1.7.2 Blanching and freezing

Freezing is widely used to preserve food products including vegetables, providing greater stability to health-promoting micronutrients such as vitamin C than drying. However, freezing causes changes in bioactive compounds, microbial counts, texture and flavor in most vegetables (Brown 1967). Fresh vegetables are normally blanched before freezing to inactivate microorganisms and prevent enzymatic activity. For seaweed, especially brown seaweed, there is an immediate transformation from brown to an attractive green color as a result of blanching. Blikra et al. (2019) compared the quality and microbial safety of fresh and frozen *Alaria esculenta* and *Saccharina latissima* blanched at different temperatures. They reported that fresh blanched seaweed was significantly greener in color than frozen unblanched seaweed for all heat treatments in both species. There were no significant differences between fresh blanched and frozen blanched *A. esculenta*, when ultimate tensile strength was used to test for texture

attributes, and low microbial counts (between 1 and 3 log CFU/g) were detected for all treatments (Blikra et al. 2019). It will be crucial to determine the effect of blanching method and product form during long term frozen storage since some of the samples in that study had a short term storage (≤ 5 days), and whether the type of blanching or seaweed product has an effect on the product quality. Another study evaluated fresh, blanched frozen, and frozen *Gracilaria*, dulse, winged kelp, and sugar kelp; and observed that the blanched samples had significantly lower total phenolic content compared to the fresh and fresh frozen samples for all four species (Nayyar 2016). Optimizing the blanching and freezing process will enhance the shelf-life and increase the revenue for the seaweed industry.

1.7.3 Rehydration

Rehydration is mostly considered for dried food products intended for direct consumption or for use in the manufacture of other products. Rehydration involves the immersion of dried food products in water or other liquids, such as fruit juices, sucrose or glucose solutions to restore some properties of the fresh product (Maldonado et al. 2010). This technique is utilized commercially for a number of dried products (e.g., instant food powder, dehydrated fruits, vegetables, and meat) that are often rehydrated or reconstituted by soaking in water prior to cooking or consumption (Rahman and Perera 2007). During rehydration, absorption of water is very fast initially, and the absorption rate decreases gradually as the moisture content approaches equilibrium. The rehydration process typically comprises of three stages that take place simultaneously including absorption of water into dried material, swelling of the rehydrated products and leaching of soluble compounds (Lee et al. 2006). There are a number of dried seaweed products

that are commercially available, hence optimizing the rehydration process for dried seaweed to maintain a relatively high product quality would be significant for chefs and consumers who prepare seaweed at home. In a seaweed study, *Himanthalia elongata* (Irish brown seaweed) was restored to original moisture content after rehydration but recorded some significant losses in phytochemical contents such as total phenolic content (83.2% loss) (Cox et al. 2012). Also, another study revealed that the impact of microelements loss in seaweed during rehydration is species dependent among some commercially available seaweeds, including *Chondrus crispus* (red seaweed), *Saccharina latissima*, *Laminaria digitata*, and *Undaria pinnatifida* (Wakame) (brown seaweeds) in Europe (Correia et al. 2021). *S. latissima* and *L. digitata* showed a more significant loss of select elements (I, Na, K, Se and tAs) as compared to the other two species during the processing steps.

1.7.4 Fermentation

Fermentation is also one of the oldest methods of food preservation and imparts desirable flavor to foods (Rolle and Satin 2002). Fermentation is a process in which chemical changes are brought about in an organic substrate through the action of free enzymes or those present in microorganisms. Fermentation could also be defined as the conversion of carbohydrates to alcohols and carbon dioxide or organic acids using yeasts, bacteria, or a combination thereof, under anaerobic conditions. Fermented foods are considered major dietary constituents in many countries because they are cost effective and contribute to food security (Rolle and Satin 2002). Various raw materials including meats, cereals, vegetables, and dairy products are used in fermentation. Although fermented foods are different across the world, they were likely produced initially as a

means of preservation, and it has been readily apparent that these foods possess other desirable attributes. Compared to the raw ingredients from which they are made, fermented foods have unique flavors, textures, appearances, and functionalities (Tamang et al. 2020). Food products that contain either probiotic microbes or prebiotic fibers have been considered functional foods that can promote health and prevent diseases (Qiang et al. 2009). However, few fermented products of aquatic origin, especially seaweeds, are known and few of these fermentations are for the production of organic acid and bioactive secondary metabolites (Uchida and Murata, 2004; Uchida and Miyoshi, 2013). Seaweed may be particularly desirable for fermentation as it could extend shelf-life and early studies have shown promising high amounts of bioactive secondary metabolites in fermented seaweeds (Wang et al. 2009; Nielsen et al. 2020; Reboleira et al. 2021).

1.8 Seaweed fermentation

The paucity of information regarding seaweed fermentation may be because of the difficulty encountered in seaweed fermentation. According to Uchida and Miyoshi (2013) seaweeds contain polysaccharides that are not ideal fermentation substrates for traditional starter cultures. Some major polysaccharides in brown algae are alginate (mannuronic and guluronic acid), laminarin (Reboleira et al. 2021) and fucoidan (fucan and sulfated polysaccharides) (Holdt and Kraan 2011), with ulvan, cellulose and hemicellulose found in green algae and seagrasses (Uchida and Miyoshi 2013; Reboleira et al. 2021). Prior studies reported 52.3, 60.0 and 66.0 g/100 g dry weight of total carbohydrates in *Ulva pertusa*, *Laminaria* sp. and *Gelidium amansii*, respectively. Small amounts of fermentable sugars such as D-glucose (18.4% weight) and D-xylose (11.6%

weight) were reported in *Ulva pertusa* (Hwang et al. 2011). Similarly, polysaccharides composed of 33.3% weight of D-glucose in *Laminaria* sp. (Roesijadi et al. 2010) and <1% weight of D-glucose and D-mannose of the total carbohydrates in *Gelidium amansii* were reported (Do et al. 1997). The reported fermentable sugars in the three seaweeds were slightly lower than those of land plants, such as corn (Hwang et al. 2011), which are good raw materials for lactic acid production, an endproduct desired in some fermented foods. The three seaweeds stated above that consist of amounts of sugars such as D-galactose, D-mannitol, L-rhamnose, D-glucuronic acid, and L-fucose were used in a study to produce lactic and acetic acids using different strains of *Lactobacillus* spp. (Hwang et al. 2011). The study indicated an unusual sugar consumption pattern, and utilization of D-gluconate, D-xylose, L-rhamnose, and L-fucose produced varying ratios of L-lactic acid to acetic acid concentrations between 0 and 6 g/L by several *Lactiplantibacillus* (formerly *Lactobacillus*) strains used in fermentation. Among the *Lactiplantibacillus* species, *L. brevis* and *L. plantarum* showed higher lactic acid yield than acetic acid in both land plants and seaweeds (especially in *Laminaria* sp.). Thus, the use of the right inoculate species in lactic fermentation of seaweed is essential in food products. Irrespective of *Lactiplantibacillus* strains, fermentation of D-gluconate and L-xylose showed higher or equal acetic acid to lactic acid ratio, and L-rhamnose and L-fucose produced very low amounts of lactic and acetic acids. A high lactic acid and lactic acid bacteria (LAB) level are good quality characteristics for certain fermented foods. Thus, there is a need to consider seaweed species with the right fermentable sugars profile to yield high lactic acid and LAB population.

Researchers have used a variety of strategies to negate the low concentration of fermentable sugars. In a study of three Irish seaweeds (*Laminaria digitata*, *Saccharina latissima* and *Himanthalia elongata*) inoculated with *Lactiplantibacillus plantarum* (formerly *Lactobacillus plantarum*), results suggested that growth of lactic acid bacteria could not be sustained in raw seaweed of any species (Gupta and Abu-Ghannam 2011). However lactic acid bacteria growth did occur in heat-treated (at 95°C with an autoclave for 15 minutes before inoculation) *L. digitata* and *S. latissima*. The heat treatment caused an increase in the amount of sugars readily used by *L. plantarum*, with the highest cell population found in *L. digitata* and *S. latissima* achieving a faster fermentation time (Gupta and Abu-Ghannam 2011).

Other researchers fermented a combination of cabbage with varying levels of *Alaria esculenta* and *Saccharina latissima* (25%, 50%, 75%) into seaweed sauerkraut products using a lactic acid bacteria (LAB) starter culture (Skonberg et al. 2021). Fresh kelp and cabbage were shredded, mixed with 2% kosher salt, and inoculated with *Lactiplantibacillus plantarum* ($\sim 10^6$ CFU/g) and *Leuconostoc mesenteroides* ($\sim 10^1$ CFU/g), and fermented at ambient temperature until a pH of < 4.6 was achieved. Kelp species and incorporation levels significantly affected most variables tested in the freshly prepared sauerkraut. LAB grew fastest in the *A. esculenta* treatments, with all products reaching a pH below 4.6 within 3 days while for *S. latissima* it took up to 14 days. All treatments had high LAB populations (above 10^6 CFU/g) after day 7 of fermentation, suggesting that lactic acid was the predominant organic acid produced during fermentation. As the seaweed concentration in the sauerkraut treatments increased, sugar concentrations in the brine decreased. Another important quality parameter is the

microbial populations in fermented foods. Both coliforms and *Vibrio* spp. were detected in some sauerkraut treatments. *Vibrio* spp. was detected only in the 75% sugar kelp treatment and this could be as a result of the long time it took to ferment. The 25% and 50% sugar kelp treatments were observed to produce the most consistent and desirable products. Also, higher antioxidant capacities were detected in 50% treatment of *S. latissima* while the 25% treatments fermented more quickly in both samples (Skonberg et al. 2021).

Another study added cellulase to aid seaweed fermentation due to the higher soluble sugar content in cellulase treated tissues (Uchida et al. 2007). In the study, wakame (*Undaria pinnatifida*) powder was either salted (3.5% w/v) or not, inoculated with different strains of *Lactiplantibacillus*, and treated with cellulase (Uchida et al. 2007). *L. brevis*, *L. plantarum*, *L. casei* and *L. rhamnosus* showed high (>90%) predominance in their cultures and the presence of salt inhibited the growth of unwanted microbes. Control treatments prepared without inoculation of LAB did not show any detectable growth of acid-producing bacteria and treatments without salt grew contaminant bacteria and spoiled (Uchida et al. 2007). Results emphasize the effects of different fermentation methods on the qualities of fermented seaweed products. Further studies will help optimize these methods to achieve reproducible high quality fermented products.

1.8.1 Inoculate species

Fermentation has a positive influence on the total phenolic content and antioxidant activity of plant-based foods; however, the degree of influence depends on the species of microorganism employed (Wijayanti et al. 2017). Lactic acid bacteria

(LAB) are widely used as starter cultures in food fermentation because some of these isolates have probiotic properties that offer health-promoting effects and also play important roles in regulating the balance of microflora in the gastrointestinal tract (Ratanaburee et al. 2013). With few fermentable sugars in seaweed, organic acid fermentation, especially lactic acid fermentation, may not be optimal. Studies on microbial strains used to break down seaweed polysaccharides have been conducted, including the use of the marine strain *Fucobacter marina* to break down fucoidans that are prevalent in seaweed (Sakai et al. 2002). Uchida and Murata (2004) examined the microbiota of fermented *Ulva* spp. to obtain starter microbes for seaweed fermentation. The predominant microbes (*Levilactobacillus brevis*, *Debaryomyces hansenii* var. *hansenii*, and *Candida zeylanoides*) after fermentation suggested that fermentation can be categorized as a mixed lactic acid and ethanol fermentation. To facilitate a high level of probiotics in fermented seaweed, the choice of LAB is crucial in seaweed fermentation, although other inoculation species have been assessed for seaweed fermentation.

Another study used different LAB strains to determine which strain could reduce the presence of spoilage bacteria in fermented rehydrated *Undaria* powder (Uchida et al. 2007). The control samples prepared without the inoculation of LAB showed no detectable LAB growth and subsequently spoiled after 11 days of storage at 20 °C. This suggests that addition of an inoculate may be a necessary component to successful seaweed fermentation. *Levilactobacillus plantarum*, *L. casei*, and *L. rhamnosus* produced more lactic acid compared to the other species and no contaminants were detected in any fermented products. Inhibition of contaminants may likely be due to the low pH obtained from the production of lactic acid (Uchida et al. 2007).

There is promise for the fermentation of seaweed for the food and beverage industries, as *Levilactobacillus plantarum* DW12 was used to ferment red seaweed (*Gracilaria fisheri*) into a beverage, which resulted in significantly higher lactic acid (>7 log CFU/g) level after 6 hours of fermentation as compared to seaweed without the cultures (Hayisama-ae et al. 2014). *Laminaria digitata* and “*L. saccharina*” (currently *Saccharina latissima*) have been successfully fermented after heat treatment and inoculation with *L. plantarum* (Gupta et al. 2011a). Bruhn et al. (2019) evaluated the effects of heat treatment and inoculation (*L. plantarum*) on the sensory and nutritional quality attributes of lacto-fermented *S. latissima*. They reported that the heat-treated and inoculated *S. latissima* stabilized kelp biomass within 48 hr and had a milder flavor and odor as compared to the fresh *S. latissima*. Evaluating the consumer acceptability of these products will facilitate the optimization of these fermented products.

1.8.2 Salt content

Traditionally, fermentation proceeds in the presence of salt, which imparts flavor to the final product and decreases levels of unwanted microorganisms in conjunction with acid produced during the process. A study reported unpublished preliminary results of unacceptable odor in wakame (*Undaria pinnatifida*) fermented without salt (Uchida et al. 2007), and different ratios of salt resulted in different product quality (Uchida et al. 2007). In the study, 2.5-3.5% salt concentration enhanced LAB growth (1.5×10^7 – 3.3×10^8 CFU/mL) during fermentation of wakame (*U. pinnatifida*) powder together with inoculate and cellulase, as compared to treatments without salt. Higher salt concentrations (5%) limited the growth of LAB. Fermented product was spoiled due to

growth of unwanted bacteria in the control samples without addition of salt. Hence, the presence of salt helped to inhibit unwanted microorganisms (Uchida et al. 2007).

1.8.3 Fermentation time

Fermentation time depends on the use or quantity of starter culture added to a substrate and the desired final pH of the product. Length of fermentation has a significant impact on some physicochemical and microbial properties of the product. The effect of fermentation time was studied when *Levilactobacillus plantarum* DW12 was used as a starter culture in a functional fermented red seaweed beverage (Ratanaburee et al. 2011). *Gracilaria fisheri* was fermented for sixty days and the effect of fermentation period on the production of lactic acid, total acid, sugar consumption, and pH levels was assessed. Results indicated that most biochemical changes occurred within the first 7 days. Maximum levels of lactic acid bacteria were achieved within the first ten days of the fermentation period and declined gradually afterwards, which correlated negatively with pH. Total sugars and bacteria counts negatively correlated with total acidity after day 7, when total sugars decreased rapidly. Although pH of the final product decreased from a range of 5 – 7 to 3.2 – 3.8 after 60 days, the largest pH change occurred within the first day of fermentation (Ratanaburee et al. 2011). When sugar kelp was mixed with cabbage at various ratio and fermented into sauerkraut, pH was not affected by sugar kelp concentration however, lactic acid increased over time during fermentation until a pH of less than 4.6 was achieved on day 14 (Skonberg et al. 2021). Therefore, monitoring pH during fermentation is vital in developing new food products not only to create uncondusive environments for pathogens, such as *Clostridium botulinum*, but to produce good product quality with high lactic acid content.

1.9 Food safety issues in the U.S.

Every year, 1 in 10 people globally become ill from eating contaminated food, resulting in up to about 420,000 deaths (WHO 2020a). These food safety issues vary across geographical areas and in the types of agents that are responsible (WHO 2020b). In the United States, there are about 50 million cases of non-specified foodborne disease every year which equates to roughly 15% of the population being sickened (CDC 2019). It has been estimated that another 9.4 million cases of foodborne illness result from known pathogens each year, with over 120,000 hospitalizations (CDC 2011). While most cases of foodborne disease are of unknown origin, a large number of cases originate from improper handling in the home and can be prevented with good sanitation and food handling practices (Clayton et al. 2003; Scallan et al. 2011; Shapiro et al. 2011). A further complication is the potential for food contamination which can occur at any stage in the food supply chain from microbiological, chemical or physical hazards. It is therefore important to fully understand safety risks associated with new foods entering the marketplace, including seaweed.

While our focus is on the microbial contamination of seaweed, there are some concerns regarding the chemical contamination of seaweed for consumption. The practice of using seaweed as an algicide in controlling blooms (Jeong et al. 2000) and in biosorption for the removal of heavy metals (e.g., arsenic, cadmium, chromium, cobalt, copper, lead, mercury) from contaminated waters is increasing (Bilal et al. 2018; Kim et al. 2019a) because it is an eco-friendly and an economical treatment process. Examples include the use of *Gracilariopsis lemaneiformis* and *Saccharina japonica* in co-cultured

farming with aquatic animals to reduce nutrient concentrations such as phosphorus, ammonium and nitrite in the water (Wu et al. 2015), and the use of *Ulva lactuca* for water bioremediation (Elizondo-González et al. 2018). Additionally, other anthropogenic activities could increase the levels of heavy metals in waters where seaweeds are cultivated, which raises concerns about impacts to consumers since there are no set maximum residue levels (MRLs) of heavy metals in seaweed in the U.S. (Kim et al. 2019a). For instance, cadmium levels detected in wakame, ogonori and kombu (1.69–1.80 mg/kg dw), and nori and *U. lactuca* (0.683–0.709 mg/kg dw) across Europe (Besada et al. 2009) exceeded MRL for cadmium in seaweed (0.5 mg/kg dw) set by the French regulation (Holdt and Kraan 2011).

1.9.1 Microbial contamination of seaweed

Microbial pathogens that can contaminate product during production or processing are a major concern about seaweed safety. Some of these pathogens, including *Vibrio* spp., are ubiquitous and persist in brackish and marine waters and have been isolated from the coastal environment of most continents (Huaishu et al. 1998; Bier et al. 2015; Jacobs Slifka et al. 2017). Mostly, outbreaks of *Vibrio* spp. normally occur in tropical or subtropical climates, although some outbreaks (*V. parahaemolyticus*) have been recorded in temperate regions such as Alaska (McLaughlin et al. 2005). A study reported an increase in occurrences of vibriosis from *V. parahaemolyticus* in Japan as ocean temperatures rise (Mahmud et al. 2007). In the study, bacteria counts increased by more than 50% in the summer as compared to winter in seawater and several seaweeds, some of which are commonly consumed as food. In the U.S., a raw frozen seaweed imported from the Philippines (tropical region), was implicated as a food vehicle for

cholera (Vugia et al. 1997). The patient showed symptoms of cholera after consumption and a nontoxigenic *V. cholerae* non-O1 isolate was later detected in leftover seaweed after an enrichment protocol by the California Department of Health Services (Vugia et al. 1997). A study indicated that the Gulf of Maine has been warming faster in the last five years than the majority of global marine waters (Pershing et al. 2021), hence this temperature change could facilitate increased overall population and seasonal prevalence of some bacterial pathogens, especially *Vibrio* spp. Recently, *V. parahaemolyticus*, *V. alginolyticus* and *Escherichia coli* were detected through enrichment and PCR techniques in seaweed (kelp samples) and estuarine waters in Maine, U.S. (Barberi et al. 2019). Even though sample materials were taken from areas not approved for bivalve aquaculture in Maine, U.S., the detection of these pathogens on seaweeds indicate a high possibility of seaweed contamination if stringent measures are not taken in regulating seaweed production.

Moreover, there are other pathogens that have been associated with seaweed contamination including *Salmonella* spp. and noroviruses. Notably, shredded dried laver seaweed (Kizami nori) was detected to be the source of four food poisoning outbreaks involving ten schools in Japan (Somura et al. 2017). Out of the number of people who consumed contaminated seaweed, 28.3% (1,193) had symptoms of gastroenteritis from Norovirus GII. The same pathogen was isolated in both the patients and shredded samples examined by real-time RT-PCR when traced back. Out of the 1,193 victims, 265 cases were tested and 207 (78.1%) tested positive for Norovirus GII. Of the 31 shredded dried seaweed samples tested, 7 (22.6%) were positive for Norovirus GII (Somura et al. 2017). Likewise, a 2012 cohort study in two schools in South Korea showed that

seasoned green seaweed (*Ulva* spp.) with radishes was significantly associated with an outbreak of gastroenteritis. Norovirus GII.6 was detected from cases from the two schools and green seaweed samples from the company that supplied the schools. In addition, Norovirus isolated from both schools was phylogenetically indistinguishable (Park et al. 2015). Although kitchen environment, storage bowls, kitchen knives, chopping boards, and dish cloths were not tested for viral pathogens, they were negative for bacterial pathogens in both schools. However, green seaweed and seawater used for washing product collected near the company were positive for Norovirus GII.6, suggesting viral pathogen contamination was from the source of seaweed production.

Fifteen cases of salmonellosis were identified in October 2017 in Hawaii and 13 cases reported consuming limu poke – a dish comprising of raw fish and seaweed a week before onset. After tracing back all food eaten, seaweed was traced back to a single aquaculture farm in Oahu, Hawaii, where an enzyme-linked fluorescent assay was used to detect *Salmonella enterica*, serovar Weltevreden in seaweed (1 out of 12) and water (10 out of 36) samples (Nichols et al. 2017). Sickesses were traced to the consumption of these contaminated seaweeds that came from the contaminated aquaculture farm. These incidents suggest that seaweeds were mostly contaminated at the production sites, hence regulations to govern the safe production of these products are necessary to ensure the safety of consumers.

1.10 Food safety regulations for seaweed in the U.S.

Regulations have been set up by various U.S. governmental institutions to establish food safety systems to ensure the safety of diverse foods. The goal of food

safety regulations is to implement a set of written documents that is based on food safety principles and incorporates Hazard Analysis and Critical Control Point (HACCP), and/or preventive controls (PC) principles. In the U.S., the Food and Drug Administration (FDA) requires the implementation of HACCP plan for some food sectors including seafoods and juice to provide food safety guidance for the industry (FDA 1997). The FDA regulates seaweed as a GRAS (generally recognized as safe) food under the category of spices (FDA 2001), however there is no guidance related to the consumption of seaweeds in larger amounts as sea vegetables. Although the production and processing of seaweeds are not covered by HACCP, the Connecticut Department of Agriculture, Bureau of Aquaculture (the lead state regulatory agency for aquaculture) requires all seaweed producers to be trained in the development of a food safety management program that includes sanitation and the application of HACCP principles to seafood processing (Concepcion et al. 2020). Recently, the FDA required all food sectors, which includes seaweed, to have food processing facilities of applicable scale to establish a food safety plan that includes an analysis of hazards and risk-based preventive controls to minimize or prevent the identified hazards (FDA 2018a).

1.10.1 Preventive controls for consumption of seaweed

In the U.S., the Food Safety Preventive Controls Alliance (FSPCA) is a broad-based public-private alliance consisting of key industry, academic, and government stakeholders whose mission is to support safe food production. The FSPCA has developed a nationwide core curriculum, training, and outreach programs to assist companies producing human and animal food in complying with the preventive controls regulations (FDA 2018a, b). These comprise of hazard analysis, supply-chain programs

and a recall plan, and delineate the procedures to be followed for monitoring, corrective actions and verification (Concepcion et al. 2020). Seaweed producers and processors of applicable business size across the nation are required to follow these guidelines (FDA 2018a).

1.11 Research needs

Seaweed aquaculture is developing rapidly in the U.S., contributing about 97% of all seaweed produced domestically (Piconi et al., 2020). However, seaweeds, especially sugar kelp, cannot be harvested throughout the year due to their short harvesting season. Moreover, seaweed has a high moisture content that can facilitate the growth of spoilage microorganisms leading to a high rate of product deterioration. Several preservation methods including drying, refrigeration, freezing, and salting, among others, have been applied to seaweed to extend its shelf-life. Refrigerating seaweed does not result in a longer extended shelf life as compared to drying or freezing, as a study reported an increase in cellular damage, texture and microbial count (reaching over 7 log CFU/g) as refrigerated storage progressed. A descriptive sensory evaluation was conducted on refrigerated samples until day 11, when samples were considered inedible (Nayyar and Skonberg 2019). For long term preservation of seaweed, drying is the most common process utilized and most of the commercialized seaweed products on the market are in the dried state. Consumers normally rehydrate dried seaweed before consumption. To the best of our knowledge, rehydration practices to ensure safety of rehydrated products have not been reported, therefore, establishing rehydration processes that will ensure the safety of dried seaweed is necessary. Notably, drying may be challenging for the seaweed

industry in temperate regions like the northern U.S., where sun drying is not available for most parts of the year. Moreover, additional cost with energy input for other forms of drying such as oven drying, and the negative impact of drying processes on labile compounds such as vitamin C in seaweed (Sappati et al. 2019) adds to the challenges of drying seaweed. However, there is an increased demand for raw and minimally processed foods including vegetables and sea vegetables of perceived quality advantages (Hollis et al. 2020). It is therefore crucial to evaluate the effect of alternative preservation methods or minimal processing methods, such as blanching and freezing, on the quality of seaweed. Although freezing is not readily used as compared to drying, optimizing the freezing processes may be beneficial to the seaweed industry in the U.S., especially when several pre-freezing procedures including blanching, are used and their impact on seaweed quality is known. Also, these minimal processes, such as blanching, may alter some qualities of seaweed that may affect consumer liking. Studies on consumer acceptability of minimally processed seaweed are few, therefore understanding consumer acceptance for minimally processed seaweed and products made from them will help increase the marketability of seaweed. As postharvest and value-addition research and studies on seaweed are gaining much attention to increase the availability of seaweed and develop innovative dishes for American consumers, the safety of seaweed should not be compromised. Since there are no guidelines established to govern the safe growing and processing of these products in most parts of the U.S., there is the need to conduct microbial challenge studies on seaweed. Results from these studies will provide a foundation to safeguard seaweed safety and augment the guidelines set up in the state of Connecticut in the U.S. to govern the production of seaweed. Therefore, research is

needed to assess the impacts of some minimal processing methods including blanching, freezing, and fermentation on the physiochemical, microbial, and sensory qualities of seaweed and in addition, to assess the safety of seaweed during storage.

1.12 Objectives

The general aim of this research was to evaluate the impact of minimal processing methods such as blanching, freezing, fermentation and rehydration on the safety and quality of sugar kelp (*Saccharina latissima*) for the development of innovative products. This will be important to the farmed seaweed industry, and seaweed processors seeking to diversify their products and needing a potential alternative to fresh seaweed. The specific objectives were:

1. To evaluate pre-freezing blanching procedures and the effects of one year of frozen storage on the physicochemical properties and microbial qualities of sugar kelp (*Saccharina latissima*) after thawing. Results will help to optimize the pre-freezing procedures required to produce high-quality products for foodservice and retail distribution, and for further value-added processing.
2. To evaluate the impacts of blanching, freezing and fermentation on the physicochemical, microbiological and sensory quality of *Saccharina latissima*. Results will provide insight into the interaction of minimal processing effects and their impact on consumer acceptability of seaweed products.
3. To determine the effect of rehydration conditions on the physicochemical and microbial properties of *Saccharina latissima*. This information will help seaweed

processors and consumers to make rehydration choices that will result in a relative higher product quality in seaweed.

4. To evaluate the survival of four pathogens inoculated on raw *Saccharina latissima* subjected to different post-harvest storage temperatures. Results will guide seaweed farmers, processors and consumers to establish procedures that will promote the safety of seaweed.

CHAPTER 2

EFFECTS OF PRE-FREEZING BLANCHING PROCEDURES ON THE PHYSICOCHEMICAL PROPERTIES AND MICROBIAL QUALITY OF FROZEN SUGAR KELP

This chapter was published in *Journal of Applied Phycology* and has undergone minor edits according to the dissertation format for consistency (Akomea-Frempong et al. 2021a).

2.1 Introduction

Seaweed is a well-known traditional food in eastern Asia. However, in Europe and North America, it is commonly processed into food additives, biofuels, and medicinal products (Rajapakse and Kim 2011; Tiwari and Troy 2015). Recently, a rapid surge in seaweed used directly for culinary purposes has been observed in the West, reportedly due in part to its numerous nutritional benefits. Edible seaweed is a source of health-promoting macro- and micro-nutrients, such as dietary fiber, omega-3 fatty acids, polyphenols, and vitamins A, B, C, and E (Rajapakse and Kim 2011; Forster and Radulovich 2015; Cherry et al. 2019).

Global production of seaweed biomass exceeds 34 million tons fresh weight and farm-raised seaweed was recently valued at over US\$ 11 billion, with an expectation of 8-12% growth per year (FAO 2020). Kelp species are the most harvested type of seaweed for human food (Buschmann et al. 2017). Despite the impacts of climate change and overharvesting on the abundance and quality of wild seaweeds (Wernberg et al. 2013; Filbee-Dexter et al. 2016), kelp have a relatively fast recovery rate and are more resilient

than most other brown seaweeds to fluctuations in the water temperature associated with global warming (Wernberg et al. 2013; Krumhansl et al. 2016). A substantial amount of seaweed cultivation focuses on kelp species, especially *Saccharina latissima* (sugar kelp), due to its high biomass yields within a short period and rich phytochemical content, which has antioxidant (Wang et al. 2010) and anti-allergenic properties (Fleurence and Ar Gall 2016). Kelp are a good source of vitamins and minerals, especially iodine, which is essential for thyroid health (Brown et al. 2014). Kelp also contain proteins that bind with zinc, chromium, and iron, forming metalloproteins (Mišurcová et al. 2011), and alginic acid, a soluble fiber that has been found to aid in weight loss (Georg Jensen et al. 2013; Brown et al. 2014). *S. latissima* has an umami-rich flavor and is attractive for food applications on its own as a sea vegetable, or as a food ingredient or flavor enhancer (Chapman et al. 2015). Producing more kelp for human consumption can provide health benefits to consumers and represents a positive step toward global food security with significant ecological and economic importance (Forster and Radulovich 2015; Kim et al. 2017).

In the U.S. and Europe, kelp species including *Alaria esculenta* (winged kelp) and *Saccharina latissima* are increasingly cultivated (Ferdouse et al. 2018). These seaweed crops are seasonal and highly perishable due to their high moisture content (Sappati et al. 2019). Established post-harvest processes for these kelp species are limited, which may limit their shelf-life and availability throughout the year for food and product development.

Drying was one of the earliest techniques developed for food preservation and is still commonly used in the preservation of kelp (Kendall et al. 2012; Fudholi et al. 2014).

However, it can present a challenge in temperate zones, including Europe and North America, where solar drying can be time-consuming and forced-air drying requires substantial energy input. Furthermore, there are negative effects of heat during drying such as diminishing the functional properties, bioactive compounds, and antioxidant activity of seaweeds including kelp (Costa et al. 2015; Neoh et al. 2016). In contrast, freezing represents an alternative preservation method to increase the availability of high-quality seaweed throughout the year, either for direct food use or further value-added processing.

Freezing provides convenience and better maintains the flavor, texture, and nutritional value of many food products compared to other long-term preservation methods (Li and Sun 2002; Tucker 2015). Although freezing retards the growth of pathogens and spoilage microorganisms (Jay et al. 2005; Tucker 2015), some deterioration in physicochemical characteristics may occur during frozen storage which may lessen food quality (De Ancos et al. 2000; Tucker 2015). In kelp (*Laminaria ochroleuca*) stored at -24 °C, counts of natural microflora were not significantly different between raw and frozen samples but L* and b* values decreased significantly after frozen storage for 180 days (del Olmo et al. 2019). Another study on frozen kelp revealed smaller changes in color of *Undaria pinnatifida* when stored at -30 °C as compared to -10, -20, and -40 °C for 60 days, however, the textural quality of the kelp significantly deteriorated (Choi et al. 2012). Freezing and frozen storage adversely affect the texture of food products due to ice crystal formation and ongoing enzymatic activity (Li and Sun 2002; Paciulli et al. 2015). A high freezing rate geared towards the production of smaller ice crystals (Li and Sun 2002) and processing methods such as blanching that inactivate

enzymes can help address some of these textural problems (Puupponen-Pimiä et al. 2003; Nilsson et al. 2004).

Blanching is a process whereby food products are briefly exposed to hot water or steam, and the process is commonly used to reduce quality deterioration in vegetables during frozen storage (DeSouza and Eitenmiller 1988; Puupponen-Pimiä et al. 2003). In broccoli, blanching at lower temperatures (60 – 65 °C) for less than 90 seconds increased firmness compared to higher temperature (70 – 90 °C) and longer blanching time (>90 seconds) (Barrett et al. 2000). The total phenolic content (TPC) of six out of eight tropical green vegetables increased significantly as compared to the unblanched samples when held in boiling water (100 °C) for 5 minutes (Oboh 2005). Likewise, a variety of blanching conditions have been evaluated for preservation or quality enhancement of seaweeds (Susanto et al. 2017; Blikra et al. 2019). Establishing blanching and freezing parameters that will reduce the deterioration rates of fresh seaweed quality will be beneficial to the industry.

Previous blanching and freezing studies reported variable effects on kelp quality (Susanto et al. 2017; Blikra et al. 2019). The ultimate tensile strength of blanched (vacuum packed, 95 °C/15 min), frozen (24 hrs), and fresh (raw) *Saccharina latissima* were not significantly different (Blikra et al. 2019), whereas the same processing parameters significantly reduced the ultimate tensile strength of blanched frozen *Alaria esculenta* as compared to raw products. Moreover, a higher blanching temperature and shorter time (85 °C/5 s) resulted in greener color in *A. esculenta* than a lower blanching temperature and longer time (54 °C/2 min), while the same blanching parameters did not significantly affect color change in *S. latissima* (Blikra et al. 2019). Nielsen et al. (2020)

blanched *S. latissima* directly in water at 30, 45, 60, and 80 °C for 2, 30, 120, and 300 s before freezing at -20 °C for 8 hrs. Ash content decreased as blanching time increased except for in kelp blanched at 80 °C, while higher blanching temperature and longer time (80 °C/300 s) significantly increased TPC compared to raw samples.

Various blanching methods have been used on sugar kelp, including direct immersion (Nielsen et al. 2020) and vacuum packaging before blanching however, a direct comparison of these methods on the quality of kelp intended for human consumption is lacking. Moreover, the freezing of seaweed without prior blanching, intended to satisfy consumers following a raw food diet, may affect product quality. Likewise, differences in product form (e.g. kelp noodles, slaw, whole blades) may have a significant effect on the quality of the frozen seaweed. To the best of our knowledge, no previous studies have reported on the impacts of product form and blanching method on kelp quality. It is essential to establish pre-freezing blanching procedures that will maintain the desired quality properties of edible seaweed and minimize its deterioration during frozen storage. The objectives of this study were to determine the effects of blanching procedures (method, temperature, and time) on the physicochemical and microbiological properties of shredded and whole blade sugar kelp during frozen storage. Results will offer food processors fundamental information for the preservation of fresh kelp and diversification of seaweed products in the market throughout the year.

2.2 Materials and methods

2.2.1 Experimental material and design

Fresh, cultivated sugar kelp (*Saccharina latissima*) harvested at commercial maturity stage in spring 2018 from Sorrento, Maine (USA) was used in this study. The study employed a partial 2^4 design to evaluate the effects of product form (whole blade, shredded slaw), blanching method (direct water immersion, vacuum package), blanching temperature (80, 100 °C) and blanching time (5, 30 seconds) on kelp quality (Figure 2.1).

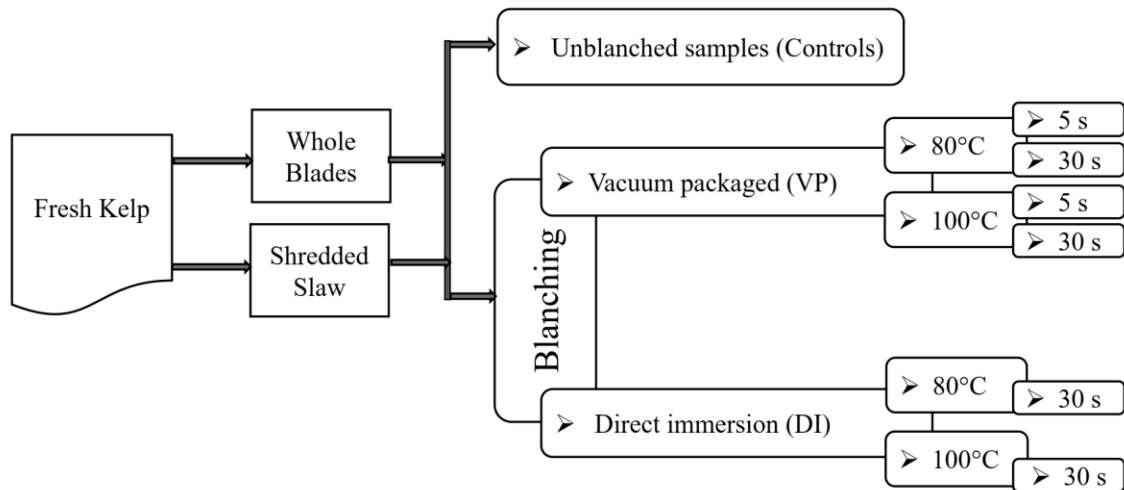


Figure 2.1: Pre-freezing processing of sugar kelp treatments

All analyses were performed on day 1, month 3, 6, 9 and 12 of frozen storage except total phenolic content (TPC), ferric reducing antioxidant power (FRAP) and selected mineral contents (calcium, magnesium, potassium and sodium). TPC and FRAP were analyzed only after 12 month of frozen storage because samples from the other testing days were not extracted and stored properly. No significant differences were detected in ash content between day 1 and month 12 samples, thus samples from testing days in between were not analyzed for ash content.

2.2.2 Sample preparation

About 78 kg of sugar kelp was harvested, washed with seawater, and delivered in coolers on ice. Samples were hand-sorted to remove debris and decayed blades before rinsing with tap water. Half of the samples were shredded with a food processor (RobotCoupe, CL 50 Series E, Jackson, MS, USA) fitted with a 1/8" slicing disc to produce shreds ranging from ~ 2–5 mm in width and ~5–25 cm in length. Approximately 350 g each of shredded slaw and whole blades were randomly sampled as raw starting material. Kelp samples were vacuum sealed under 99% vacuum in 12 in x 12 in plastic bags (Ultrasource, Kansas, MO) before blanching (vacuum packaged) or after blanching (direct immersion) and prior to analyzing for physicochemical and microbial properties.

2.2.3 Blanching

Kelp samples for each treatment replicate were weighed (350 g/batch) and blanched by direct immersion or after vacuum sealing (KOCH Ultravac, Model UV550, USA) in plastic bags (Ultrasource, USA). Direct immersion (DI) and vacuum packaged (VP) samples were placed in metal strainers and held in a 50-L steam-jacketed kettle about ¾ full of hot water for the prescribed time/temperature combinations. The temperature of the water was monitored with a thermocouple (Omega, Stamford, CT) throughout the process. Internal temperature of the vacuum sealed bags was not measured for experimental consistency with the direct immersion samples, which could not be directly monitored for “internal” temperature. After blanching, the samples were immediately cooled in an ice/water slurry (~ 1 °C) for 1 min, with direct immersion samples subsequently transferred into sample bags and subjected to vacuum packaging.

2.2.4 Storage conditions

After blanching, samples were immediately blast frozen (Southeast Cooler, Lithia Springs, GA) at -30°C for an hour and then stored at -20°C for up to 1 year. Samples were subjected to physicochemical and microbial analyses after 1 day, 6 months, and 12 months of frozen storage. Unblanched kelp samples stored at -20°C were used as controls. All frozen samples were thawed overnight at $\sim 5^{\circ}\text{C}$ prior to analysis.

2.2.5 Drip loss

Drip loss was assessed to determine how much tissue fluids were lost from the seaweeds during storage. Drip loss was measured by draining and weighing all the tissue fluids present in each sample bag after thawing through a hole made in the plastic bag. The bag was tilted for about one minute to decant the liquid. Drip loss was calculated as percent fluid lost compared to the initial sample weight using the following formula:

$$\%Drip\ loss = \left(\frac{Fluid\ loss\ (g)}{Initial\ sample\ weight\ (g)} \right) \times 100$$

2.2.6 Instrumental texture

Several methods were used to analyze the texture of the different product forms of kelp. The Kramer shear method by Johanningsmeier et al. (2007) with some modifications was used to evaluate the texture of the shredded slaw samples. Briefly, 15 g of shredded sample was loaded into a mini Kramer shear cell (TA-XTi2, Texture Technologies Inc., USA) with five flat blades set to travel 5 cm in a downward direction at a pre-test and post-test speed of 2 mm/s. The texture analyzer was calibrated using a 5,000 g load cell before each use. Force (N) required to shear the sample was recorded as the hardness of the shredded kelp. Ten subsamples from each treatment replicate were analyzed and values were averaged. The force required to shear the sample was recorded

by the texture analysis software (Exponent 32, version 5.0, 6.0, 2010, Texture Technologies Inc., Scarsdale, NY). Eight to ten subsamples from each treatment replicate were analyzed and values were averaged. For analysis of kelp blades, circular pieces of ~6-cm diameter were randomly cut with a scissors from blade samples and placed (three layers) on the texture analyzer platform. The texture analyzer was calibrated similarly as for the Kramer shear method. A flat-bottomed cylindrical probe of 5-cm diameter was used to compress the kelp blade samples with 75% strain at a pre-test and post-test speed of 2 mm/s test speed. Hardness (N), the maximum force of the first compression, was recorded by the texture analysis software (Exponent 32, version 5.0, 6.0, 2010, Texture Technologies Inc.) on 8–10 samples per treatment replicate. Resilience (regaining original height after the first compression) was calculated by dividing the upstroke energy of the first compression by the downstroke energy of the first compression. Percent softening was calculated by adapting the formula of Rinaldi et al. (2013), as shown below for day 1 samples to evaluate the effect of immediate freezing on softening. Percent softening at months 6 and 12 was calculated in comparison to day 1 hardness values to determine the effects of long-term frozen storage on softening.

$$\% \text{ softening} = \left(1 - \frac{\text{hardness (N) of kelp samples on each test day}}{\text{hardness (N) of fresh (raw) kelp}} \right) \times 100$$

2.2.7 Colorimetric analyses

Change in color of kelp during frozen storage was measured with a colorimeter (LabScan XE, Hunter Labs, USA) fitted with a 5.1-cm diameter aperture, a port size of 5.05 cm, area view of 4.45 cm, and D65 illumination. The colorimeter was standardized with white and black tiles before each use and the colorimeter was allowed to warm up

for 30 min prior to color analysis. Blades or shredded slaw were placed to cover the bottom of a transparent cup of about 60 mm in diameter with a height of 7 mm and L^* , a^* , and b^* values were determined. Ten samples were analyzed and averaged for each of the three treatment replicates. The immediate effect of blanching and frozen storage on color change (ΔE) was determined on day 1 in comparison to raw samples. Month 6 and 12 values also were compared to day 1 values to determine the long-term effect of frozen storage on ΔE using the following formula:

$$\Delta E_{ab}^* = \sqrt{(L_2^* - L_1^*)^2 + (a_2^* - a_1^*)^2 + (b_2^* - b_1^*)^2}$$

where L^* denotes lightness, a^* denotes the red (+a) to green (-a) color axis and b^* denotes the yellow (+b) to blue (-b) color axis. One (1) represents values for raw samples before frozen storage and 2 represents values of day 1 frozen samples for immediate ΔE ; whereas one (1) represents values for day 1 frozen samples and 2 represents values from other frozen storage testing days for storage ΔE .

2.2.8 Moisture and ash content

Moisture content was determined according to AOAC Method 950.46 B, by weighing approximately 5 g homogenized kelp sample in a pre-weighed aluminum pan and drying at 105 °C for 6 h (AOAC 2005a) in a convection oven (VWR International, Radnor, PA). All tests were conducted in duplicate and moisture content was expressed in g/100 g on a wet weight basis (wwb) using the formula below:

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

Ash content was also determined gravimetrically according to AOAC method 938.08 (AOAC 2005b). One gram of oven-dried sample was placed in a pre-weighed scintillation vial, charred on a hot plate set on medium until the cessation of smoke emission prior to ashing samples in a muffle oven (Thermolyne Model F-A1730, Dubuque, IA) at 550 °C for 6 h. Vials containing the samples were re-weighed and percent ash was then calculated in duplicates on a wet weight basis (wwb) as follows:

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

2.2.9 Mineral analysis

Ashed samples were dissolved in concentrated acid (HNO₃:HCl; 7:1 v/v). After the bubbling of samples had stopped, 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. About 15 mL of each sample was poured into a new pre-labelled scintillation vial and samples were then analyzed by inductively coupled plasma optical emission spectroscopy (Thermo Elemental IRIS Intrepid DUO ICP-OES, USA) to determine calcium, magnesium, potassium, and sodium content. All the samples were analyzed in triplicate and reported in g/100g on a wet weight basis (wwb).

2.2.10 Antioxidant analysis

2.2.10.1 Sample preparation

Samples were freeze-dried (VirTis Ultra, Warminster, PA, USA) using multiple 30h drying cycles until the samples reached a constant weight. The freeze-dried samples were ground using a coffee grinder (Hamilton Beach Fresh Coffee Grinder, USA), and stored at -80 °C until extraction for antioxidant analysis. Freeze-dried samples (2 g) were

mixed with 20 mL of 60% methanol (v/v) and shaken on a lab plate shaker at 210 rpm for 24 h at room temperature. The 24 h extraction time and 60% methanol concentration for extraction of polyphenols were chosen based on preliminary tests of a previous study in our laboratory, which maximized extraction of polyphenols (Nayyar 2016). The mixture was centrifuged at $2100 \times g$ (Beckman Avanti J-25, Brea, CA) for 10 min and the supernatant was collected. The pellet was washed twice with 10 mL of 60% (v/v) methanol, followed by vortexing for 30 s and centrifuging at $2100 \times g$ for 10 min. All supernatants from the extraction and pellet wash were collected and then brought to a final volume of 50 mL with deionized water. The extracts were stored at -20°C prior to conducting total phenolic content (TPC) and ferric reducing antioxidant power (FRAP) assays.

2.2.10.2 Total phenolic content (TPC) assay

Total phenol content was determined in duplicate using the Folin-Ciocalteu reagent according to the method of Sappati et al. (2019), with slight modifications. Briefly, Folin-Ciocalteu was diluted with distilled water (1:10). Then, 1.5 mL of diluted Folin-Ciocalteu was added to 0.2 mL of methanolic kelp extracts. After a five-minute incubation period, 1.5 mL of 6% sodium bicarbonate solution was added and the mixture was agitated vigorously. The samples were then placed in the dark for 1 hour at room temperature (22°C). The absorbance of the samples was read at 725 nm using a UV-vis spectrophotometer (Beckman Du 530, Brea, CA) against a 42% methanol blank of varying concentrations of gallic acid (0-200 $\mu\text{g/mL}$) as a standard. Results were expressed as mg of gallic acid equivalent (GAE) per gram of freeze-dried sample. Analyses were run in duplicate and the values were averaged per treatment replicate.

2.2.10.3 FRAP assay

The assay used was modified from Benzie and Strain (1996). FRAP reagents were prepared fresh daily by mixing sodium acetate buffer (300 mM), 10 mM 2,4,6-tripyridyl-s-triazine (TPTZ), and 20 mM ferric chloride ($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$) in the ratio (10:1:1). The solution was stirred and warmed to 37 °C in a water bath before 3 mL of 37 °C FRAP reagent was added to 0.1 mL of sample and the 50 – 750 μM ferrous sulfate ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$) standard. After 4 min, the absorbance was determined at 593 nm using a UV-vis spectrophotometer (Beckman Du 530, Brea, CA) against a deionized water sample blank. A standard curve comprising of 50 – 750 μM ferrous sulfate ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$) and an internal control of 250 μM Trolox in 42% MeOH was used. All samples were analyzed in duplicate and results were expressed as μmol ferrous sulfate equivalents (FSE) per gram of freeze-dried sample.

2.2.11 Microbiological analysis

Microbial safety analysis was performed on raw (fresh) seaweed samples before frozen storage. Methods of detection for *Vibrio* spp., *Listeria monocytogenes*, *Salmonella* spp. and *Staphylococcus aureus* were modified from the U.S. FDA's Bacteriological Analytical Manual (FDA, 2018c). Briefly, 25 g of each of the samples were placed aseptically into 225 mL of pathogen-specific broth (Table 2.1), placed in a stomacher bag and homogenized for two minutes using a BAGMixer 400 (Model P, Spiral Biotech, Advanced Instruments, Norwood, MA, USA). Afterward, the stomacher bag was incubated for the prescribed time and samples were plated (0.1 mL) onto pathogen-specific plates in duplicate for each of the three treatment replicates. The presence of

colony growth with expected morphology denoted the presumptive presence of pathogens.

Table 2.1: Media and incubation conditions for microbial analysis of sugar kelp

Microorganism	Enrichment Broth	Agar Medium
<i>Vibrio</i> spp.	Alkaline peptone water (28 °C for 24 hrs)	Thiosulfate-citrate-bile salts-sucrose agar (28 °C for 48 hrs)
<i>Listeria monocytogenes</i>	Listeria enrichment broth (28 °C for 24 hrs)	Modified oxford agar base (28 °C for 48 hrs)
<i>Salmonella</i> spp.	Lactose broth (35 °C for 24 hrs)	Xylose lysine deoxycholate agar (35 °C for 48 hrs)
<i>Staphylococcus aureus</i>	Tryptic soy broth with 10% NaCl and 1% sodium pyruvate (35 °C for 24 hrs)	Baird-Parker (35 °C for 48 hrs)

Aerobic plate count (APC), fungi, and psychrotrophs were enumerated in kelp across frozen storage. Ten grams of each of the kelp treatments were aseptically placed in a stomacher bag with 90 mL of 0.1% peptone (BD Diagnostics, USA) and stomached for two minutes using a BAGMixer 400. For APC, serial dilutions in 0.1% peptone were plated in duplicate on tryptic soy agar (TSA) (Alpha Biosciences, Baltimore, MD) for each of the three treatment replicates. TSA plates were inverted and incubated for 48 hours at 37 °C. Plates within the countable range (20-200 colonies) were counted. Duplicate values for each treatment replicate were averaged, and the data were reported as log colony forming units (CFU) per gram. The same process was repeated for psychrotrophs except that TSA plates were incubated for 10 days at 4 °C. Similarly, serial dilutions in 0.1% peptone were plated in duplicate on acidified potato dextrose agar (APDA) comprised of potato dextrose agar (Alpha Biosciences, Baltimore, MD) with

10% tartaric acid (final pH 3.5) to ensure the growth of fungi. Plates were incubated at ambient temperature (20 °C) for 5 days and plates with 15 to 150 colony-forming units were enumerated.

2.2.12 Statistical analysis

IBM SPSS Statistics 20 was used to analyze recorded data. One way analysis of variance (ANOVA) was used to assess all one-level (treatment) effects. Outliers were removed using a 3 X Interquartile range (IQR) procedure. Multi-way analysis of variance (MANOVA) was used to determine any significant effects ($P < 0.05$) of the independent variables (product form, blanching method, blanching temperature, blanching time, and frozen storage time) on the response variables (physicochemical and microbiological properties). Tukey's Honest Significant Difference (HSD) test was selected for post hoc analyses. Independent T-tests were used to analyze immediate effects of blanching and freezing between raw samples and day 1 frozen samples. Pearson's correlation was performed to evaluate correlations among dependent variables.

2.3 Results and discussion

Additional results and discussion that were not included in the published paper are presented in Appendix A.

2.3.1 Texture and drip loss

2.3.1.1 Whole blades

The overall model effect shows that blanching method and blanching time did not significantly impact any of the texture attributes of the whole blades, whereas blanching temperature affected the resilience of the blades (Table 2.2). Higher blanching

temperature significantly preserved blade resilience as compared to lower blanching temperature, but blanching temperature did not affect hardness or chewiness of the whole blades. When comparing raw kelp blades to the day one blanched frozen kelp blade treatments (Table 2.3), independent t-tests showed no immediate effects of blanching and freezing on hardness or chewiness. These findings are similar to the results of a prior blanching/freezing study on sugar kelp (85 °C/5 s, 24 hrs; Blikra et al., 2019). However, when considering the long-term effects of frozen storage, the hardness, chewiness, and resilience of kelp blades decreased significantly ($P < 0.05$) in most treatments after 12 months (Table 2.3). These changes were progressive as frozen storage time increased. The significant decrease in resilience for all blanched kelp blades after 12 months of frozen storage was the most notable textural change observed with regard to the expected impact on consumer acceptance. A decrease in resilience may affect the “stronger bite” descriptor used for sugar kelp (Bruhn et al. 2019).

Table 2.2: Model effect (*P*-values) on the qualities of sugar kelp during 12 months frozen storage

Dependent variables	Whole blades				Shredded slaw			
	Blanching method	Blanching temperature	Blanching time	Frozen storage	Blanching method	Blanching temperature	Blanching time	Frozen storage
Color								
L*	< 0.001	0.071	0.565	0.003	< 0.001	0.407	0.480	0.007
a*	< 0.001	0.015	0.096	< 0.001	< 0.001	0.055	0.406	0.012
b*	< 0.001	< 0.001	0.572	0.021	< 0.001	< 0.001	0.805	0.576
Texture								
Hardness	0.513	0.559	0.441	< 0.001	0.081	0.072	0.393	0.267
Chewiness	0.934	0.807	0.806	< 0.001				
Resilience	0.411	0.007	0.098	< 0.001			N/A	
Chemical & Physical								
Moisture	< 0.001	0.685	0.561	0.764	0.001	0.187	0.131	0.625
% Drip loss	0.011	0.490	0.521	0.099	0.067	0.472	0.793	0.150
Ash	< 0.001	0.836	0.072	0.442	0.156	0.755	0.243	0.074
Calcium	0.008	0.762	0.254	0.601	0.068	0.526	0.657	0.715
Magnesium	0.642	0.686	0.765	0.321	0.001	0.403	0.525	0.579
Potassium	< 0.001	0.930	0.015	0.288	0.001	0.670	0.149	0.274
Sodium	< 0.001	0.955	0.046	0.091	0.004	0.427	0.239	0.897
TPC	< 0.001	< 0.001	< 0.001	N/A	< 0.001	0.006	0.350	N/A
FRAP	< 0.001	0.722	0.132	N/A	< 0.001	0.691	0.852	N/A
Microbial								
APC	0.191	0.354	0.629	0.860	0.665	0.523	0.995	0.661
Psychrotrophs	-	-	-	-	0.021	0.261	0.015	0.470
Fungi	0.886	0.121	0.384	0.450	0.415	0.698	1.000	0.170

Bold numbers: Significant, N/A = Not analyzed, - = No results generated after statistical analysis

Interactions: No results for 4-ways and some 3-ways interactions and too many 2-ways interactions to be shown on table (Additional results are presented in Appendix B).

Drip loss is crucial in frozen vegetables since essential water-soluble chemical constituents can be lost when vegetables are subjected to a freeze-thaw cycle. Similarly, a study reported that thawing frozen *Saccharina latissima* resulted in drip loss equivalent to almost half of the raw material wet weight, which consisted of over 90% water and a small amount of dry matter including minerals, phenolic compounds and proteins (Sund 2020). In the present study, drip losses of up to about 25% of the raw material wet weight were observed in all frozen samples (Table 2.3 & 2.4). Only the blanching method (DI or VP) had a significant impact on drip loss of whole blades (Table 2.2), while blanching temperature and blanching time did not. All blanched samples had significantly higher drip loss on day one as compared to raw kelp blades, but were not significantly different from unblanched frozen controls at any time point (Table 2.3). As frozen storage progressed, VP blanched samples exhibited significantly higher drip loss than DI samples. The significant impact of blanching method on drip loss was likely due to the plastic pouch used in the VP blanching process, which retained any liquid released from the kelp during blanching and frozen storage. In contrast, any cellular fluid lost during DI blanching was released to the blanching water. Moreover, due to the enveloping plastic pouch, it is possible that the maximum internal product temperature during VP blanching may have been lower than in the DI samples, potentially allowing undenatured enzymes in the VP samples to break down kelp cell walls to release more cellular fluid during long term frozen storage. The use of a thermocouple to monitor internal product temperature during the VP blanching process is recommended for future studies to more clearly understand the impact of product temperature on kelp quality. Nonetheless, vacuum

packaged blanching may be recommended for convenience and verifiable, uniform temperature control when handling kelp in a processing environment.

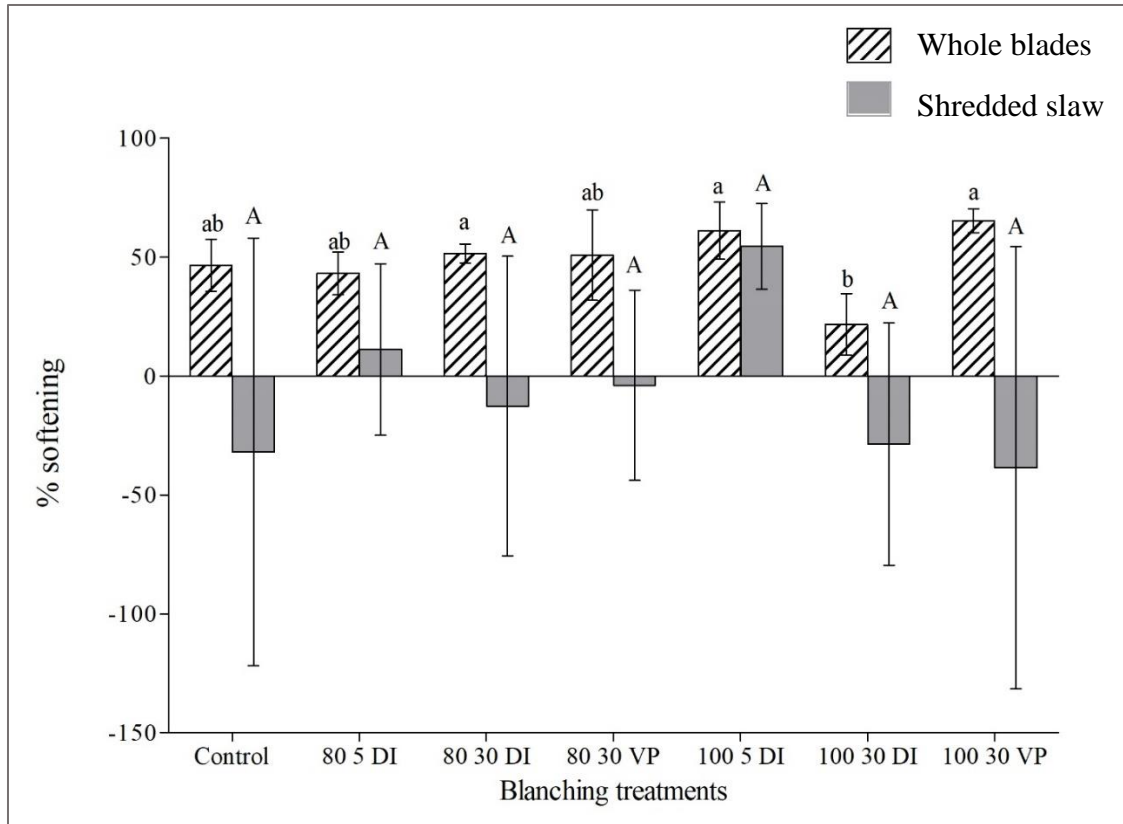
Table 2.3: Texture and drip loss in whole blade sugar kelp during 12 months frozen storage [mean \pm SD (n = 3)]

Storage Time	Texture parameters		Hardness (N)		Chewiness		Resilience		% Drip loss	
	Blanching procedures									
Day 1	Raw		230.7	± 42.5	164.0	± 31.1	0.88	± 0.05	4.0	± 2.4
	Unblanched		211.0	± 46.5 ^{aA}	99.3	± 28.3 ^{aA}	0.75	± 0.03 ^{aB}	17.3	± 7.7 ^{aA}
	DI	80 °C 5s	203.4	± 60.7 ^a	100.1	± 43.8 ^a	0.79	± 0.02 ^{aB}	18.9	± 4.7 ^{aA}
		80 °C 30s	240.2	± 83.1 ^{aA}	118.8	± 56.7 ^a	0.76	± 0.02 ^{aA}	17.8	± 2.2 ^a
		100 °C 5s	245.4	± 25.5 ^{aA}	120.9	± 50.9 ^{aAB}	0.80	± 0.01 ^{aB}	18.4	± 8.9 ^a
		100 °C 30s	177.4	± 96.6 ^a	86.9	± 59.4 ^a	0.79	± 0.03 ^{aA}	15.3	± 8.9 ^a
M6	VP	80 °C 30s	248.2	± 59.9 ^{aA}	133.2	± 49.5 ^a	0.79	± 0.03 ^{aB}	19.0	± 0.9 ^a
		100 °C 30s	242.1	± 60.1 ^{aA}	132.6	± 51.5 ^{aA}	0.79	± 0.01 ^{aB}	24.3	± 6.1 ^a
	Unblanched		179.4	± 24.4 ^{aAB}	142.9	± 11.3 ^{abcAB}	0.88	± 0.04 ^{aA}	15.8	± 10.9 ^{aA}
	DI	80 °C 5s	175.4	± 27.5 ^a	124.9	± 32.8 ^{abc}	0.83	± 0.01 ^{abA}	10.6	± 11.8 ^{aA}
		80 °C 30s	209.1	± 14.2 ^{aAB}	144.3	± 6.2 ^{abc}	0.79	± 0.02 ^{bA}	12.4	± 3.7 ^a
		100 °C 5s	214.6	± 28.7 ^{aA}	168.4	± 14.4 ^{aA}	0.86	± 0.02 ^{abA}	13.4	± 2.7 ^a
100 °C 30s		174.4	± 29.7 ^a	118.4	± 14.6 ^{bc}	0.87	± 0.05 ^{abA}	12.2	± 1.6 ^a	
M12	VP	80 °C 30s	206.2	± 9.5 ^{aAB}	158.5	± 7.6 ^{ab}	0.83	± 0.00 ^{abA}	19.6	± 8.0 ^a
		100 °C 30s	153.4	± 10.1 ^{aAB}	108.8	± 18.3 ^{cAB}	0.84	± 0.03 ^{abA}	15.0	± 8.6 ^a
	Unblanched		112.5	± 23.3 ^{aB}	58.6	± 19.5 ^{aB}	0.01	± 0.00 ^{aC}	5.0	± 2.3 ^{aB}
	DI	80 °C 5s	115.5	± 19.9 ^a	54.4	± 22.7 ^a	0.00	± 0.00 ^{aC}	6.6	± 5.1 ^{aB}
		80 °C 30s	116.3	± 9.9 ^{aB}	63.2	± 17.1 ^a	0.01	± 0.00 ^{aC}	9.1	± 5.6 ^a
		100 °C 5s	95.2	± 30.4 ^{aB}	48.5	± 27.8 ^{aB}	0.01	± 0.00 ^{aC}	17.9	± 14.0 ^a
100 °C 30s		139.0	± 23.9 ^a	73.8	± 20.3 ^a	0.01	± 0.00 ^{aC}	8.7	± 9.0 ^a	
VP	80 °C 30s	121.8	± 49.3 ^{aB}	62.4	± 47.3 ^a	0.01	± 0.00 ^{aC}	20.4	± 8.3 ^a	
	100 °C 30s	83.7	± 12.8 ^{aB}	45.1	± 14.9 ^{aB}	0.01	± 0.00 ^{aC}	19.1	± 7.9 ^a	

M6 = Month 6, M12 = Month 12, DI = Direct immersion, VP = Vacuum packaged, s = seconds.

Superscripts: different small letters indicate significant difference among treatments within a test period & capital letters show significant difference within a specific treatment across 12 months frozen storage (one-way ANOVA). Absence of capital letters indicates no significant differences during storage.

Blanching method, temperature, and time had no significant effect on percent softening of whole blades (Figure 2.2). However, the interaction between blanching method and frozen storage duration was significant, where DI induced a higher immediate percent softening as compared to VP in day 1 samples. A minimal impact of blanching and freezing was observed on the mean percent softening on day one (1.7%), indicating that the applied blanching parameters and overnight frozen storage did not significantly soften the texture of the whole blades as compared to the raw product. To determine the long term effect of frozen storage on whole blade texture, percent softening at months 6 and 12 were calculated in comparison to day 1 samples. Percent softening for month 6 samples (15.8%) was significantly lower than for month 12 samples (49.0%), suggesting that post-blanching frozen storage of more than 6 months may adversely affect the hardness of kelp blades.



Control = Unblanched kelp, DI = Direct immersion, VP = Vacuum packaged, 80 = 80 °C, 100 = 100 °C, 5 = 5 seconds, 30 = 30 seconds. Letters indicate significant difference across treatments (one-way ANOVA): small letters within whole blades and capital letters within shredded slaw treatments.

Figure 2.2: Effect of blanching treatments on percent softening in sugar kelp after 12 months of frozen storage in comparison to day 1 [mean \pm SD (n = 3)]

2.3.1.2 Shredded samples

Blanching method, temperature, time and frozen storage duration had no significant model level effect on the hardness of shredded kelp (Table 2.2). Likewise, the individual blanching treatments and duration of frozen storage had no significant immediate or long-term effect on shredded kelp hardness (Table 2.4). The lack of significant treatment effects was likely due to the high standard deviations recorded during texture analysis as a result of the high heterogeneity of the shredded slaw. The

high standard deviations may also have been contributed by insufficient sample mass placed in the mini Kramer shear cell during texture analysis. The analysts' approach emphasized subsample quantity (n=10) rather than subsample mass (15 g) to minimize variability in the shredded slaw shear data, but future analyses should evaluate the impacts of increased sample mass on reducing standard deviations in this heterogeneous product.

Table 2.4: Texture and drip loss in shredded slaw sugar kelp during 12 months frozen storage [mean \pm SD (n = 3)]

Storage Time	Blanching Procedures		Shear force (N) ‘hardness’			% Drip loss		
Day 1	Raw		52.3	±	19.3	6.9	±	1.8
	Unblanched		42.8	±	29.1	16.4	±	2.8
	DI	80 °C 5s	28.6	±	21.9	14.3	±	3.0
		80 °C 30s	37.1	±	27.6	12.0	±	1.3
		100 °C 5s	46.3	±	28.0	13.0	±	11.7
		100 °C 30s	31.2	±	4.5	16.6	±	13.7
		80 °C 30s	36.8	±	15.8	15.9	±	8.3
	VP	100 °C 30s	42.7	±	18.1	21.8	±	7.6
		Unblanched	43.0	±	24.9	15.2	±	7.0
	M6	DI	80 °C 5s	20.5	±	6.9	7.6	±
80 °C 30s			31.0	±	15.9	8.2	±	4.8
100 °C 5s			30.4	±	25.6	7.4	±	6.0
100 °C 30s			25.9	±	4.6	8.2	±	7.2
VP		80 °C 30s	33.6	±	15.5	13.5	±	8.3
		100 °C 30s	31.7	±	19.6	10.6	±	7.8
Unblanched		56.5	±	38.9	16.4	±	9.3	
M12		DI	80 °C 5s	25.4	±	10.5	6.5	±
	80 °C 30s		41.8	±	23.6	7.1	±	3.1
	100 °C 5s		21.0	±	8.6	12.2	±	5.6
	100 °C 30s		40.1	±	16.1	12.7	±	9.1
	VP	80 °C 30s	38.2	±	14.8	16.1	±	5.3
		100 °C 30s	59.2	±	39.9	15.7	±	5.6

M6 = Month 6, M12 = Month 12, DI = Direct immersion, VP = Vacuum packaged, s = seconds.
Absence of superscript indicates no significant differences during storage.

Blanching method, temperature and time, and duration of frozen storage had no significant effect on drip loss or on percent softening (Table 2.2, Figure 2.2). Percent drip loss in shredded slaw remained fairly constant or decreased over storage time, although not significantly. The mean percent softening for shredded slaw on day 1 was 27.0%, as compared to 1.7% for the whole blades, indicating a substantially higher immediate impact of blanching and freezing on the slaw than on whole blades, likely due to the mechanical disruption of cells and subsequent release of exudate in response to shredding. However, the mean percent softening values for shredded slaw on month 6 and month 12 of frozen storage were not significantly different from each other or from day 1 samples, suggesting that shredded slaw may better preserve its texture during long term frozen storage in contrast to whole blades which experienced an increase in softening from month 6 to month 12 of frozen storage. Although different texture analysis methods were used for whole blades and shredded slaw, percent softening measures the rate of change and not the unit magnitude, allowing indirect comparison of textural changes in the whole blade and shredded slaw samples. Nonetheless, the high variability in percent softening of the slaw prevents specific conclusions about the textural quality of shredded kelp in comparison to whole blades during long term frozen storage. However, consumers may prefer shredded samples to whole kelp blades because of their convenience for use in home food preparation.

2.3.2 Color

Product form had no statistically significant effect on color, therefore data for whole blades and shredded slaw were pooled and analyzed together, with mean values reported in Table 2.5. There were no significant differences in color (L^* , a^* and b^*

values) of unblanched samples on day 1 as compared to raw samples (Table 2.5), indicating no effect of overnight freezing on kelp color. However, blanching method, temperature and long term frozen storage had a significant model effect on L^* , a^* and b^* values (Table 2.2). Direct immersion blanching and a higher blanching temperature (100 °C) significantly increased L^* and b^* values, and decreased a^* values as compared to vacuum packaged blanching and lower blanching temperature (80 °C). As frozen storage progressed, mean Hunter a^* and b^* values increased and decreased, respectively. These changes in L^* , a^* , and b^* values represent a brighter and greener coloration in all blanched frozen samples compared to raw kelp samples. However, L^* and a^* values increased as frozen storage prolonged, indicating further lightening and loss of green color during frozen storage. Also, from month 6 onwards, samples blanched by direct immersion demonstrated significantly higher a^* values compared to vacuum packaged samples, representing a more severe loss of green coloration growing more pronounced as frozen storage continued.

Table 2.5: Color (Hunter L*, a*, b*) of sugar kelp (both product forms) during 12 months frozen storage [mean \pm SD (n = 3)]¹

Blanching Procedures			L*		a*		b*		ΔE		
D1	Raw		17.5	\pm 1.3	3.3	\pm 1.6	11.8	\pm 1.7	--	\pm	--
	Unblanched		15.8	\pm 1.6 ^{dB}	2.9	\pm 0.4 ^a	12.3	\pm 2.9 ^c	2.5	\pm	0.9 ^d
	DI	80 °C 5s	21.9	\pm 2.5 ^{abc}	-1.8	\pm 1.7 ^{cd}	19.8	\pm 3.1 ^b	10.7	\pm	3.1 ^{ab}
		80 °C 30s	24.2	\pm 2.1 ^a	-3.7	\pm 0.7 ^{dB}	26.2	\pm 3.5 ^a	7.1	\pm	0.9 ^{bcd}
		100 °C 5s	23.8	\pm 1.6 ^{ab}	-3.1	\pm 0.9 ^{dB}	26.3	\pm 1.6 ^a	13.5	\pm	2.5 ^{aA}
		100 °C 30s	22.6	\pm 3.0 ^{abc}	-2.1	\pm 1.2 ^d	18.5	\pm 1.7 ^{bB}	8.0	\pm	0.6 ^{bcA}
	VP	80 °C 30s	18.6	\pm 1.3 ^{cdB}	1.6	\pm 0.8 ^{ab}	14.8	\pm 2.7 ^{bc}	13.7	\pm	0.6 ^{aA}
		100 °C 30s	20.0	\pm 2.9 ^{bc}	0.0	\pm 1.3 ^{bc}	17.9	\pm 3.5 ^b	3.5	\pm	1.6 ^{cdB}
6	Unblanched		18.0	\pm 1.5 ^{bAB}	3.1	\pm 1.1 ^a	14.4	\pm 3.0 ^d	3.3	\pm	2.5 ^c
	DI	80 °C 5s	23.1	\pm 1.7 ^a	-1.1	\pm 1.5 ^b	21.3	\pm 1.0 ^{bc}	9.6	\pm	3.4 ^{ab}
		80 °C 30s	24.2	\pm 0.7 ^a	-2.0	\pm 0.4 ^{bA}	25.3	\pm 1.7 ^{ab}	4.5	\pm	1.7 ^{bc}
		100 °C 5s	25.0	\pm 1.7 ^a	-1.9	\pm 0.4 ^{aA}	26.5	\pm 3.5 ^d	12.5	\pm	1.2 ^{aAB}
		100 °C 30s	23.4	\pm 1.8 ^a	-1.6	\pm 0.7 ^b	25.1	\pm 2.6 ^{aA}	4.4	\pm	0.9 ^{bcB}
	VP	80 °C 30s	20.1	\pm 1.3 ^{bAB}	2.3	\pm 0.4 ^b	15.8	\pm 0.9 ^{ab}	11.3	\pm	1.9 ^{aAB}
		100 °C 30s	20.1	\pm 1.1 ^b	1.5	\pm 0.6 ^b	18.0	\pm 2.0 ^{cd}	8.4	\pm	1.2 ^{abcA}
	Unblanched		21.0	\pm 2.9 ^{bA}	3.3	\pm 0.6 ^a	15.0	\pm 3.4 ^c	5.2	\pm	2.3 ^{abc}
12	DI	80 °C 5s	22.6	\pm 1.6 ^{ab}	-0.3	\pm 1.5 ^{cd}	19.0	\pm 2.4 ^{abc}	5.8	\pm	2.6 ^{ab}
		80 °C 30s	25.4	\pm 1.4 ^a	-1.4	\pm 0.3 ^{dA}	23.0	\pm 2.3 ^a	5.1	\pm	3.4 ^{abc}
		100 °C 5s	24.3	\pm 1.2 ^{ab}	-1.1	\pm 0.7 ^{dA}	23.1	\pm 1.7 ^a	8.3	\pm	1.4 ^{aB}
		100 °C 30s	24.0	\pm 1.9 ^{ab}	-1.0	\pm 1.4 ^d	21.9	\pm 2.6 ^{aAB}	1.9	\pm	1.0 ^{cC}
	VP	80 °C 30s	21.3	\pm 1.8 ^{bA}	2.1	\pm 0.7 ^{ab}	16.1	\pm 2.1 ^{bc}	8.8	\pm	2.2 ^{aB}
		100 °C 30s	22.7	\pm 1.9 ^{ab}	1.3	\pm 1.6 ^{bc}	20.6	\pm 3.5 ^{ab}	4.0	\pm	1.8 ^{abcB}

Table indicates pooled average of shredded slaw and whole blade kelp

M6 = Month 6, M12 = Month 12, DI = Direct immersion, VP = Vacuum packaged, s = seconds.

Superscripts: different small letters indicate significant difference among treatments within a test period & capital letters show significant difference within a specific treatment across 12 months frozen storage (one-way ANOVA). Absence of capital letters indicates no significant differences during storage.

Hunter (L*, a*, b*): L* = lightness, a*= red/green, b*=yellow/blue, ΔE =Change in color

Color is important for consumer acceptance of fresh vegetables (Barrett et al. 2000), likewise, for sugar kelp. Results of this study indicate that blanching using different methods, temperatures and times significantly influenced the color of *Saccharina latissima*. L^* values increased as a^* values decreased, likely as a result of the breakdown of brown fucoxanthin pigments during heat treatment (Zhao et al. 2019). According to Silva and Silva (1999), a ΔE value of 0.5 – 1.5 represents a small change in color, 1.5 – 3.0 represents a distinct change, 3.0 – 6.0 represents a very distinct change, 6.0 – 12.0 denotes a great alteration and values above 12 indicate a very great color transformation. Color change was distinct ($\Delta E > 1.5$) in the unblanched kelp frozen for 24 hours as compared to raw kelp, indicating that other factors such as light in addition to heat processing contributed to the breakdown of fucoxanthin in kelp (Zhao et al. 2019), as indicated in a previous study (Susanto et al. 2017). The freezing and thawing process also may have impacted the color of kelp, but further study is warranted to support that conclusion. However, the significantly higher ΔE values observed in blanched samples as compared to unblanched samples on day 1 suggest that thermal processing degraded fucoxanthin more than other factors. After freezing, the color of blanched kelp samples remained unchanged regardless of the blanching temperature and time throughout six months of frozen storage (Table 2.5). Similarly, in a prior study, the color of blanched and frozen (24 hrs) *Saccharina latissima* remained relatively constant at a specific temperature regardless of the blanching time in the range of 1 s up to 15 min (Blikra et al. 2019). In the current study, significantly higher a^* values were recorded in samples exposed to lower blanching temperature (80 °C) and shorter time (5 s) after 12-month storage, suggesting that a higher blanching temperature and longer blanching time prior

to frozen storage may be preferred. Also, when comparing the blanched frozen samples on month 12, VP blanched samples were darker (lower L^* values) and redder (higher a^*) than DI samples but similar to unblanched kelp on month 12. These results indicate that direct immersion, a higher blanching temperature, and a longer blanching time produced a brighter green color irrespective of the product form. However, the significant increase in a^* values in DI samples between month 6 and 12 represents a loss of green coloration that might negatively affect kelp marketability. Long-term frozen storage resulted in a few treatments exhibiting a significant decrease and increase in L^* and a^* values at 12-months, respectively (Table 2.5). Although samples were stored in the dark, the change in L^* and a^* values indicate that there are other factors that can degrade carotenoids (such as the green-hued xanthophyll, fucoxanthin) in kelp, apart from exposure to light (Hii et al. 2010).

2.3.3 Moisture, ash, and selected mineral contents

Product form and blanching temperature had no significant impact on moisture, ash, sodium, or potassium levels in the samples (Table 2.2), but product form significantly affected calcium and magnesium contents, with higher calcium and magnesium contents detected in the whole blade treatments as compared to shredded slaw. Blanching time only affected potassium levels in the kelp blades (Table 2.2), where the short blanching time (5 s) reduced the potassium levels in whole blades significantly as compared to the longer blanching time. Moreover, there were no significant immediate effects of blanching and freezing on moisture, sodium and potassium levels of whole blades and shredded slaw (Table 2.6 & 2.7), as seen in the day 1 samples as compared to the fresh raw kelp samples. Blanching significantly increased mean moisture content of

the kelp compared to the unblanched control, while direct immersion further increased the moisture content significantly as compared to vacuum packed blanched and unblanched samples. VP increased the ash content, potassium and sodium levels in samples as compared to DI blanched samples. Moisture, ash, and mineral contents remained unchanged during frozen storage.

Table 2.6: Moisture, ash and selected minerals in whole blade sugar kelp during 12 months frozen storage [mean \pm SD (n = 3)]

Storage Time	Blanching procedures	Moisture (g/100g)	Ash (% wwb)	Calcium (g/100g wwb)	Magnesium (g/100g wwb)	Potassium (g/100g wwb)	Sodium (g/100g wwb)
Day 1	Raw	88.6 \pm 0.9	6.0 \pm 2.7	0.32 \pm 0.02	0.23 \pm 0.04	1.62 \pm 0.44	0.42 \pm 0.09
	Unblanched	88.5 \pm 0.7 ^a	5.5 \pm 0.5 ^{aA}	0.22 \pm 0.01 ^a	0.12 \pm 0.02 ^a	1.57 \pm 0.80 ^a	0.40 \pm 0.23 ^a
	DI	80 °C 5s	92.3 \pm 1.0 ^a	3.4 \pm 0.6 ^b	0.22 \pm 0.04 ^a	0.12 \pm 0.01 ^a	1.12 \pm 0.22 ^a
		80 °C 30s	92.1 \pm 1.2 ^a	2.8 \pm 0.4 ^b	0.27 \pm 0.02 ^a	0.13 \pm 0.01 ^a	0.78 \pm 0.15 ^a
		100 °C 5s	91.1 \pm 3.1 ^a	2.8 \pm 0.5 ^b	0.24 \pm 0.03 ^a	0.14 \pm 0.03 ^a	1.06 \pm 0.61 ^a
		100 °C 30s	92.1 \pm 1.4 ^a	2.7 \pm 0.4 ^b	0.26 \pm 0.04 ^a	0.13 \pm 0.03 ^a	0.67 \pm 0.16 ^a
	VP	80 °C 30s	88.9 \pm 1.0 ^a	5.0 \pm 0.2 ^a	0.20 \pm 0.06 ^a	0.13 \pm 0.01 ^a	1.52 \pm 0.22 ^a
		100 °C 30s	89.0 \pm 0.1 ^a	5.4 \pm 0.4 ^a	0.22 \pm 0.03 ^a	0.13 \pm 0.01 ^a	1.77 \pm 0.16 ^a
M12	Unblanched	89.0 \pm 3.5 ^a	3.9 \pm 0.3 ^{abcB}	0.23 \pm 0.04 ^a	0.12 \pm 0.01 ^a	1.57 \pm 0.47 ^a	0.36 \pm 0.04 ^{ab}
	DI	80 °C 5s	92.1 \pm 0.8 ^a	3.3 \pm 0.1 ^{abc}	0.24 \pm 0.03 ^a	0.14 \pm 0.01 ^a	1.07 \pm 0.02 ^{abc}
		80 °C 30s	91.9 \pm 2.1 ^a	2.2 \pm 1.9 ^c	0.26 \pm 0.03 ^a	0.13 \pm 0.02 ^a	0.75 \pm 0.20 ^{bc}
		100 °C 5s	91.9 \pm 1.2 ^a	3.0 \pm 0.9 ^{abc}	0.25 \pm 0.02 ^a	0.13 \pm 0.03 ^a	0.75 \pm 0.32 ^{bc}
		100 °C 30s	92.9 \pm 0.6 ^a	2.5 \pm 0.3 ^{bc}	0.23 \pm 0.03 ^a	0.12 \pm 0.01 ^a	0.62 \pm 0.10 ^c
	VP	80 °C 30s	88.0 \pm 2.7 ^a	4.9 \pm 0.4 ^{ab}	0.22 \pm 0.02 ^a	0.12 \pm 0.00 ^a	1.49 \pm 0.24 ^{ab}
		100 °C 30s	89.9 \pm 1.4 ^a	5.0 \pm 0.9 ^a	0.20 \pm 0.04 ^a	0.12 \pm 0.01 ^a	1.69 \pm 0.38 ^a

M12 = Month 12, DI = Direct immersion, VP = Vacuum packaged, s = seconds.

Superscripts: different small letters indicate significant difference among treatments within a test period & capital letters show significant difference within a specific treatment across 12 months frozen storage (one-way ANOVA). Absence of capital letters indicates no significant differences during storage.

Table 2.7: Moisture, ash and selected minerals in shredded slaw sugar kelp during 12 months frozen storage [mean \pm SD (n = 3)]

Storage Time	Blanching procedures	Moisture (g/100g)	Ash (% wwb)	Calcium (g/100g wwb)	Magnesium (g/100g wwb)	Potassium (g/100g wwb)	Sodium (g/100g wwb)
Day	Raw	88.5 \pm 0.4	5.7 \pm 2.1	0.27 \pm 0.05	0.18 \pm 0.02	1.18 \pm 0.21	0.40 \pm 0.05
	Unblanched	88.5 \pm 0.0 ^c	5.3 \pm 0.7 ^a	0.20 \pm 0.00 ^a	0.12 \pm 0.00 ^a	1.49 \pm 0.23 ^a	0.40 \pm 0.04 ^a
	80 °C 5s	92.9 \pm 0.2 ^{ab}	2.6 \pm 0.6 ^{bc}	0.21 \pm 0.01 ^a	0.11 \pm 0.01 ^a	0.73 \pm 0.04 ^a	0.20 \pm 0.05 ^a
	DI 80 °C 30s	93.6 \pm 0.4 ^a	2.2 \pm 0.4 ^c	0.20 \pm 0.05 ^a	0.10 \pm 0.01 ^a	0.56 \pm 0.05 ^a	0.16 \pm 0.02 ^a
	100 °C 5s	93.9 \pm 0.2 ^a	2.5 \pm 0.5 ^c	0.19 \pm 0.03 ^a	0.11 \pm 0.02 ^a	0.73 \pm 0.30 ^a	0.18 \pm 0.08 ^a
	100 °C 30s	92.6 \pm 1.7 ^{ab}	4.9 \pm 2.1 ^{abc}	0.19 \pm 0.02 ^a	0.11 \pm 0.02 ^a	1.16 \pm 0.91 ^a	0.27 \pm 0.21 ^a
	VP 80 °C 30s	89.0 \pm 1.7 ^c	4.9 \pm 0.3 ^a	0.25 \pm 0.04 ^a	0.13 \pm 0.01 ^a	1.63 \pm 0.21 ^a	0.38 \pm 0.13 ^a
	100 °C 30s	90.2 \pm 1.6 ^{bc}	4.0 \pm 0.9 ^{ab}	0.23 \pm 0.01 ^a	0.13 \pm 0.01 ^a	1.46 \pm 0.30 ^a	0.32 \pm 0.11 ^a
	Unblanched	90.8 \pm 3.0 ^a	4.2 \pm 1.4 ^a	0.20 \pm 0.05 ^a	0.11 \pm 0.02 ^{ab}	1.40 \pm 0.56 ^{ab}	0.36 \pm 0.14 ^{ab}
	80 °C 5s	93.2 \pm 0.8 ^a	2.4 \pm 0.3 ^a	0.21 \pm 0.04 ^a	0.11 \pm 0.01 ^{ab}	0.67 \pm 0.07 ^{bc}	0.19 \pm 0.05 ^b
M12	DI 80 °C 30s	93.2 \pm 2.0 ^a	0.8 \pm 1.0 ^a	0.18 \pm 0.04 ^a	0.10 \pm 0.01 ^b	0.46 \pm 0.02 ^c	0.14 \pm 0.01 ^b
	100 °C 5s	93.6 \pm 0.6 ^a	2.0 \pm 0.2 ^a	0.21 \pm 0.01 ^a	0.10 \pm 0.00 ^{ab}	0.53 \pm 0.09 ^{bc}	0.14 \pm 0.03 ^b
	100 °C 30s	90.4 \pm 3.4 ^a	4.3 \pm 1.8 ^a	0.22 \pm 0.07 ^a	0.12 \pm 0.02 ^{ab}	1.33 \pm 0.60 ^{abc}	0.34 \pm 0.15 ^{ab}
	VP 80 °C 30s	89.5 \pm 1.0 ^a	3.6 \pm 2.9 ^a	0.23 \pm 0.06 ^a	0.14 \pm 0.01 ^a	1.65 \pm 0.18 ^a	0.47 \pm 0.12 ^a
	100 °C 30s	92.2 \pm 0.5 ^a	2.8 \pm 1.1 ^a	0.21 \pm 0.01 ^a	0.11 \pm 0.01 ^{ab}	0.97 \pm 0.17 ^{abc}	0.28 \pm 0.03 ^{ab}

M12 = Month 12, DI = Direct immersion, VP = Vacuum packaged, s = seconds.

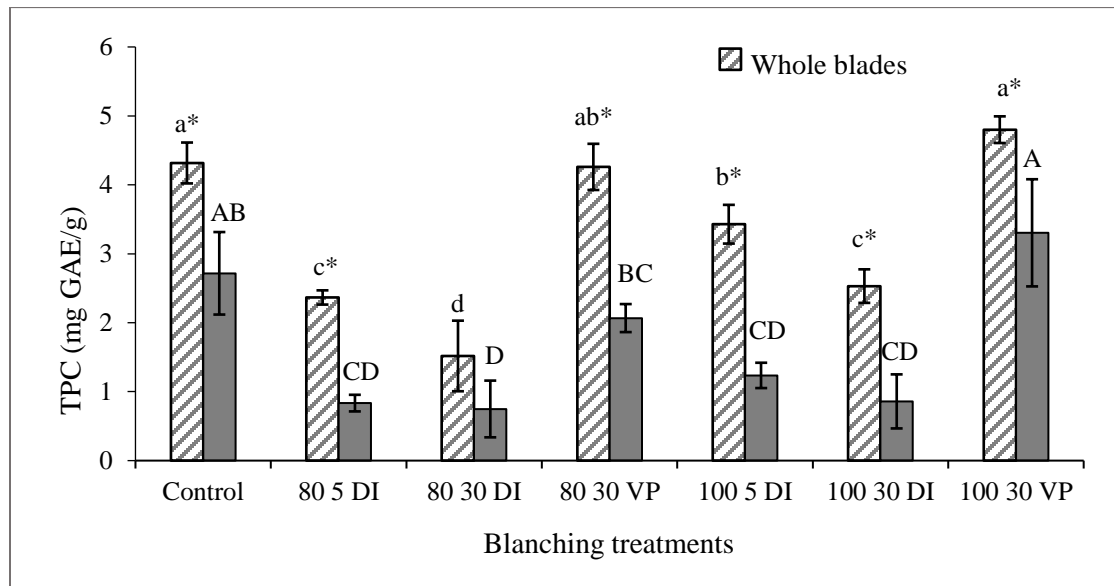
Superscripts: different small letters indicate significant difference among treatments within a test period & capital letters show significant difference within a specific treatment across 12 months frozen storage (one-way ANOVA). Absence of capital letters indicates no significant differences during storage.

The observed moisture and mineral contents of untreated fresh kelp in this study were within the ranges reported in previous studies on sugar kelp (Schiener et al. 2014; Perry et al. 2019). The decrease in ash and select minerals in some treatments (Table 2.6 & 2.7) of both blanched and unblanched frozen samples at day 1 may be a result of the high drip loss recorded. Also, any minerals present on the surfaces of the blades, or exposed during shredding, may have leached into the blanch water. This possibility is supported by the significantly higher mineral levels measured in the VP samples as compared to the DI blanched samples. Extended frozen storage did not significantly affect mineral concentrations because of the minimal drip loss observed. The reduction in ash content of blanched samples ranged from about 7-86% as compared to the ash content of raw kelp. This result is substantially different from a study by Nielsen et al. (2020) which reported no significant effect of direct immersion blanching on ash content of sugar kelp. The specific mineral levels of sugar kelp in the current study were within the ranges reported in other *Saccharina latissima* studies (Circuncisão et al. 2018), and confirm that sugar kelp is a good source of selected minerals post blanching and freezing. The levels of sodium, calcium, magnesium, and potassium in the frozen sugar kelp provided moderate to high average daily intakes (ADI) (12.2 – 32.2%, 18.9 – 27.4%, 24.8 – 55.3%, and 16.2 – 253.1%, respectively) (Meyers et al. 2006) per 100 g serving (wwb) of shredded or whole blade kelp.

2.3.4 Total phenolic content and ferric reducing antioxidant power

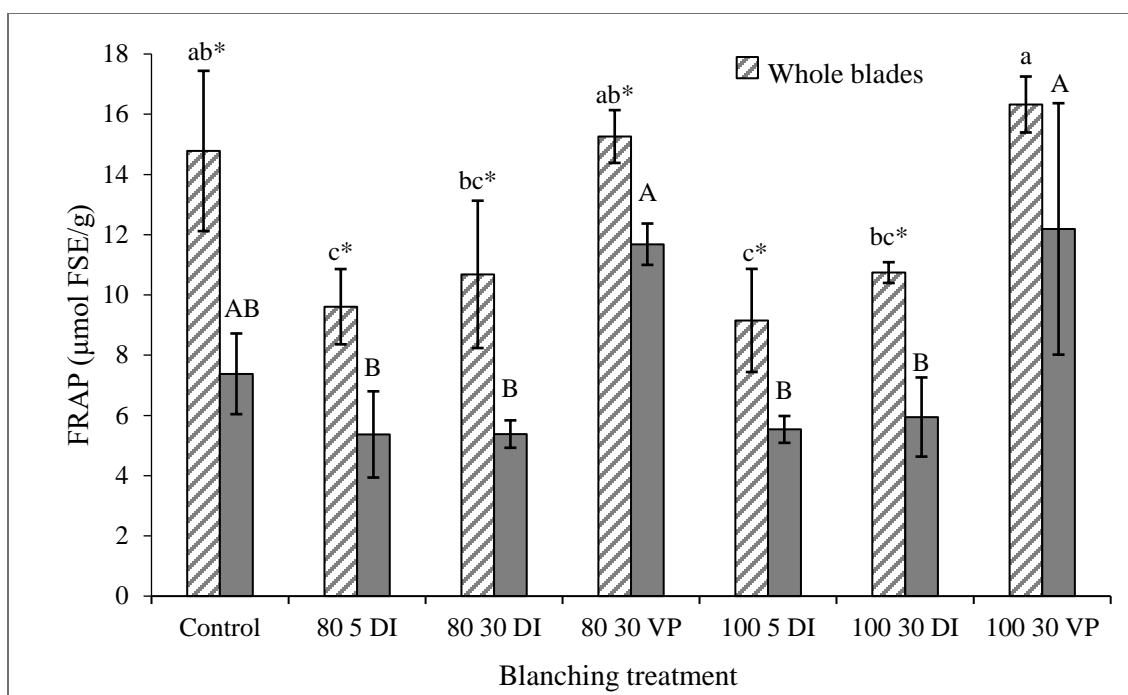
Results indicate that whole blades had significantly higher ($P < 0.05$) TPC (Figure 2.3) and FRAP values (Figure 2.4) than shredded slaw irrespective of the blanching procedure and duration of frozen storage. Blanching method, temperature, and

time did not have significant effects on FRAP values but did significantly impact TPC (Table 2.2). The higher blanching temperature, longer blanching time, and VP blanching method resulted in significantly higher TPC values in both product forms of kelp compared to other blanching parameters. The more extensive thermal processing may have destroyed the cell wall structure of kelp, leading to the release of soluble phenolic compounds (Lou et al. 2014) and facilitating their extraction and quantification. Whereas the blanching parameters may not have affected the levels of other secondary metabolites in kelp that have the ability to reduce Fe^{3+} , as measured by the FRAP assay. The interaction between the three factors above resulted in TPC values that were not significantly different from the unblanched samples (control), and after 12 months of frozen storage, TPC and FRAP values were not significantly different between the VP blanched kelp and the unblanched control.



Control = Unblanched kelp, DI = Direct immersion, VP = Vacuum packaged, 80 = 80 °C, 100 = 100 °C, 5 = 5 seconds, 30 = 30 seconds. Letters indicate significant differences across treatments (one-way ANOVA): small letters within whole blades and capital letters within shredded slaw treatments. Asterisks indicate significant difference between the two product forms within blanching treatment.

Figure 2.3: Effect of blanching treatments on total phenolic content ‘TPC’ (mg GAE/g freeze-dried wb) of sugar kelp after 12 months of frozen storage [mean \pm SD (n = 3)]



Control = Unblanched kelp, DI = Direct immersion, VP = Vacuum packaged, 80 = 80 °C, 100 = 100 °C, 5 = 5 seconds, 30 = 30 seconds. Letters indicate significant differences across treatments (one-way ANOVA): small letters within whole blades and capital letters within shredded slaw treatments. Asterisks indicate significant difference between the two product forms within blanching treatment.

Figure 2.4: Effect of blanching treatments on ferric reducing antioxidant power ‘FRAP’ (μmol FSE/g freeze-dried wb) in sugar kelp after 12 months of frozen storage [mean ± SD (n = 3)]

Blanching is recognized to reduce phenolic content in vegetables and seaweeds (Puupponen-Pimiä et al. 2003; Susanto et al. 2017), as was observed in the DI blanching treatments. The VP blanching method likely better retained the phenolic compounds and secondary metabolites that act as antioxidants in the kelp whereas they leached into the blanching water in the DI method. In other reports, blanching at higher temperatures resulted in the loss of more phenolic compounds from vegetables and seaweeds, including kelp (Steinberg 1995; Oboh 2005; Susanto et al. 2017). Surprisingly, in our study, the higher blanching temperature (100 °C) resulted in higher levels of phenolic

compounds measured in kelp. However, this result is similar to the higher TPC values observed in *Saccharina latissima* subjected to blanching at 60 °C as compared to 45 °C (Nielsen et al. 2020). The authors hypothesized that the increase in TPC was a result of a concentrating effect due to the leaching of other compounds from the kelp during direct immersion blanching. The TPC values were strongly correlated with FRAP values, as expected ($r = 0.822$, $P \leq 0.01$). The strong correlation between TPC and FRAP indicates how phenolic compounds such as phlorotannins and bromophenols, and flavonoids derived from seaweeds (Kim et al. 2012) can potentially act as antioxidants by scavenging free radicals. The higher FRAP values in whole blade kelp ($\bar{x} = 12.37 \pm 2.98$ $\mu\text{mol/g}$) compared to shredded kelp ($\bar{x} = 7.64 \pm 3.02$ $\mu\text{mol/g}$) make it a superior source of antioxidants as compared to other blanched frozen vegetables such as green peas (*Pisum sativum* L.), which had an average FRAP value of 0.61 ± 0.22 $\mu\text{mol/g}$ (Nilsson et al. 2004).

2.3.5 Microbial safety and quality

Vibrio spp, *Listeria monocytogenes*, *Salmonella* spp. and *Staphylococcus aureus* were undetectable in all fresh raw kelp received. There was no significant difference between product forms (kelp blade and shredded slaw) for microbial counts, therefore data for both product forms were pooled, analyzed, and presented in Table 2.8. A relatively low aerobic plate count (APC) was found for fresh raw blades (2.7 – 3.6 log CFU/g) and shredded slaw (2.4 – 3.4 log CFU/g), and notably, the handling involved in shredding did not increase APC levels. Blanching method, temperature, and time, as well as frozen storage, had no significant effect on APC (Table 2.2). Psychrotrophs and fungi for all treatments were consistently below 2.5 log CFU/g. Psychrotrophs on kelp

remained statistically unchanged after 12 months of frozen storage for all blanched and unblanched samples. However, the fungi count was significantly ($P < 0.05$) higher after 12 months of frozen storage (2.1 log CFU/g) as compared to other timepoints (≤ 2.0 log CFU/g), as a result of fungi (mold) recovered from unblanched samples on month 12. Mold was detected on control samples only, which statistically differentiates these samples from all other treatments and suggests a potential quality benefit from blanching.

Table 2.8: Microbial counts (mean +/- s.d.) of sugar kelp (both product forms) during 12 months frozen storage

Storage Time	Blanching procedures	Pathogens ¹	APC (Log CFU/g)	Psychrotroph (Log CFU/g)	Fungi (Log CFU/g)
	Raw	Absent	3.36 ± 0.31 ^a	2.00 ± 0.00 ^a	2.03 ± 0.42
Day	Unblanched		2.75 ± 0.82 ^a	2.00 ± 0.00 ^a	2.00 ± 0.00 ^{aB}
	80 °C 5s		2.93 ± 0.58 ^a	2.00 ± 0.00 ^a	2.04 ± 0.05 ^a
	DI 80 °C 30s		2.93 ± 0.58 ^a	2.00 ± 0.00 ^a	2.04 ± 0.05 ^a
	100 °C 5s		2.85 ± 0.37 ^a	2.00 ± 0.00 ^a	2.00 ± 0.00 ^a
	100 °C 30s		2.91 ± 0.41 ^a	2.10 ± 0.16 ^a	2.04 ± 0.05 ^a
	VP 80 °C 30s		2.96 ± 0.42 ^a	2.00 ± 0.00 ^a	2.00 ± 0.00 ^a
	100 °C 30s		3.03 ± 0.54 ^a	2.00 ± 0.00 ^a	2.00 ± 0.00 ^a
	Unblanched		2.82 ± 0.66 ^a	2.00 ± 0.00 ^a	2.04 ± 0.04 ^{aB}
	80 °C 5s		2.79 ± 0.52 ^a	2.00 ± 0.00 ^a	2.00 ± 0.00 ^a
	DI 80 °C 30s		3.18 ± 0.72 ^a	2.00 ± 0.00 ^a	2.00 ± 0.00 ^a
M6	100 °C 5s		2.85 ± 0.88 ^a	2.00 ± 0.00 ^a	2.00 ± 0.00 ^a
	100 °C 30s		3.04 ± 0.47 ^a	2.10 ± 0.16 ^a	2.00 ± 0.00 ^a
	VP 80 °C 30s		2.81 ± 0.57 ^a	2.00 ± 0.00 ^a	2.00 ± 0.00 ^a
	100 °C 30s		3.06 ± 1.17 ^a	2.00 ± 0.00 ^a	2.00 ± 0.00 ^a
	Unblanched		2.59 ± 0.54 ^a	2.00 ± 0.00 ^a	2.14 ± 0.05 ^{aA}
	80 °C 5s		2.63 ± 0.59 ^a	2.00 ± 0.00 ^a	2.00 ± 0.00 ^b
	DI 80 °C 30s		3.08 ± 1.37 ^a	2.00 ± 0.00 ^a	2.00 ± 0.00 ^b
	100 °C 5s		3.14 ± 1.45 ^a	2.00 ± 0.00 ^a	2.00 ± 0.00 ^b
	100 °C 30s		2.71 ± 0.26 ^a	2.00 ± 0.00 ^a	2.00 ± 0.00 ^b
	VP 80 °C 30s		2.82 ± 0.57 ^a	2.00 ± 0.00 ^a	2.00 ± 0.00 ^b
M12	100 °C 30s		2.65 ± 0.51 ^a	2.00 ± 0.00 ^a	2.00 ± 0.00 ^b

Values indicate pooled averages of both shredded slaw and whole blade kelp

¹Pathogens = *Vibrio* spp., *Listeria monocytogenes*, *Salmonella* spp., and *Staphylococcus aureus*

M6, month 6; M12, month 12; DI, direct immersion; VP, vacuum packaged; s, seconds

Superscripts: different small letters indicate significant differences among treatments within a test period and capital letters show a significant difference within a specific treatment across 12-month frozen storage (one-way ANOVA). Absence of capital letters indicates no significant differences during storage
CFU, coliform forming units; APC, aerobic plate count; Fungi, yeast and molds

A recent report suggests the potential for contamination of kelp with human pathogens during cultivation when water bodies in which kelp grow become contaminated (Barberi et al. 2019). Additionally, the introduction of pathogens uncommon to the marine environment, such as *Listeria monocytogenes*, during handling and post-harvesting processing into finished products (Gupta et al. 2010) could be another route for kelp contamination. However, the four pathogenic organisms tested for in this study were absent in kelp (Table 2.8). The absence of these pathogens on kelp samples may be the result of the cleanliness of the water in which these kelp are grown in Sorrento, Maine, which has a higher microbial quality as compared to the water quality in Casco Bay and Saco Bay in Maine (Barberi et al. 2019), that are not approved for shellfish harvesting by the National Shellfish Sanitation Program. Additionally, sanitary handling procedures, or the demonstrated antimicrobial activity of brown seaweed (Cox et al. 2010) may have contributed to the absence of pathogens on the kelp. Microbial counts were low throughout 12 months of frozen storage, suggesting a minimal risk of spoilage from bacteria or fungi. Although our data confirm the consistency of kelp's microbial quality during frozen storage, safety cannot be inferred from this study. Additional work is warranted to assess the efficacy of these blanching procedures against inoculated pathogens.

2.4 Conclusions

Shredded kelp slaw had significantly lower TPC and FRAP values but may be preferred to whole blades for its convenience and consistent texture during frozen storage since its other quality attributes were not negatively affected by blanching. Blanching

after vacuum packaging resulted in higher concentrations of sodium and potassium, and higher TPC and FRAP values in kelp as compared to direct immersion blanching. Higher blanching temperature (100 °C) and longer time (30 s) increased the brightness and greenness of sugar kelp, which may positively influence marketability. Consumer acceptance testing of blanched products is warranted to assess effects of color and texture changes on acceptability of sugar kelp. Future frozen storage studies should also assess the quality of sugar kelp immediately post blanching to more clearly discriminate between the impacts of blanching versus frozen storage. In summary, this study indicates that pre-freezing blanching procedures significantly influenced frozen kelp quality. Vacuum packaged blanching at 100 °C for 30 s, followed by freezing at -20 °C, resulted in color changes that may be desirable to consumers, fewer changes in textural attributes, and a higher content of selected minerals in comparison to other blanching treatments, thereby supporting its application as an effective long-term storage practice for producers to help diversify the market for sugar kelp products.

CHAPTER 3

IMPACT OF BLANCHING, FREEZING, AND FERMENTATION ON PHYSICOCHEMICAL, MICROBIAL, AND SENSORY QUALITY OF SUGAR KELP (*SACCHARINA LATISSIMA*)

This chapter was published in *Foods* and has undergone minor edits according to the dissertation format for consistency (Akomea-Frempong et al. 2021b).

3.1 Introduction

Seaweed cultivation offers potential solutions to environmental challenges, such as eutrophication, by improving water quality (Kim et al. 2015a, 2019a; Zheng et al. 2019). Seaweeds have a higher production rate than terrestrial plants, and they do not require land or fresh water (Chapman et al. 2015). The sustainability of seaweed cultivation has increased the appeal for their production through aquaculture globally. Moreover, consumers perceive edible seaweed food products as natural and healthy (Cornish et al. 2015; Roohinejad et al. 2017). Seaweeds are rich in dietary fiber, minerals, vitamins, antioxidants, and umami flavor; they can be used in low-calorie diets and serve as functional foods (Cornish and Garbary 2010; Cornish et al. 2017; Wells et al. 2017; Figueroa et al. 2021).

There are numerous seaweed-based products in Asian countries such as China, South Korea, and Japan, with niches of products marketed in Europe and North America. The FAO reported that 290,000 wet tons of seaweed were produced in 2019 in the Americas and Europe (FAO 2021). The principal cultivated variety (66%) was kelp, a grouping which encompasses multiple species of brown algae (FAO, 2017; Kim et al.,

2017). In the U.S., seaweed cultivation is found on the west and east coasts, with Maine and Alaska leading U.S. production (~85%) of about 600,000 wet lbs. of edible seaweed due to their extensive coastlines, as reported by the Island Institute in 2020 (FAO 2017; Piconi et al. 2020). The increasing production provides abundant opportunities for industrial development for seaweed consumption. However, little attention has been paid to consumers' perceptions of seaweed as a food product in the West (Lucas et al. 2019). Also, the extreme seasonality and high perishability of the crop (Perry et al. 2019; Skrzypczyk et al. 2019) may impede the availability of raw materials to produce consumer products without the use of preservation processes.

Prior studies have applied various processes, including drying, freezing, salting, and high-pressure processing, to various seaweed species to increase seaweed product availability throughout the year (Gupta et al. 2011b; del Olmo et al. 2019; Perry et al. 2019). Most of these processes reduced some bioactive compounds and changed the texture of seaweed (Choi et al. 2012; Sappati et al. 2019). Blanching prior to some of these preservation methods, including drying and freezing, has been suggested to retard product deterioration rates (Del Rosario and Mateo 2019). Moreover, blanching reduces microbial counts in some vegetables (Edgar and Aidoo 2001) and turns brown seaweed to a bright green color (Blikra et al. 2019). Processing methods such as fermentation and salting may also add value to seaweed products in addition to providing shelf-life extension.

Fermentation is a low-cost preservation method utilized by some food processors, which increases some bioactive compounds in foods such as cabbage (Drašković Berger et al. 2020), and give food products unique flavor (Paramithiotis 2017). Seaweeds can be

fermented into a seaweed sauerkraut-style products to create a non-dairy alternative probiotic product for consumers (Gupta et al. 2012; Skonberg et al. 2021). Sugar kelp (*Saccharina latissima*) and winged kelp (*Alaria esculenta*) mixed with cabbage in various ratios were fermented with *Lactobacillus plantarum* (10^6 CFU/g) and *Leuconostoc mesenteroides* (10^1 CFU/g) starter cultures to produce seaweed sauerkraut with high lactic acid bacteria levels, which increased as fermentation progressed (Skonberg et al. 2021). Fermentation of sugar kelp with *L. plantarum* for 48 hours reduced mercury and cadmium content significantly ($P < 0.05$), as compared to raw kelp (Bruhn et al. 2019), which could relieve concerns about heavy metals for health-conscious consumers.

To develop appropriate food products for western markets from the harvest of domestic seaweeds and also consider seaweed as a vegetable, it is crucial to consider cost-effective preservation methods such as blanching, freezing, and fermentation, which can extend the shelf-life of the raw materials. In the literature available to date, studies on assessment and consumer acceptance of minimally processed seaweed food products are limited. Recent work conducted in our laboratory showed that blanching of sugar kelp resulted in significant changes immediately after treatment, including differences in physicochemical properties of kelp (compared to unblanched samples), particularly color and texture, after 12 months of frozen storage (chapter two). These significant changes in some of the kelp qualities in response to blanching and/or frozen storage may have a measurable effect on consumer acceptance and may influence commercialization of blanched and/or frozen seaweed food products. Therefore, the hypothesis of this paper was that blanching, freezing, and fermentation may increase kelp quality and consumer acceptability. The effect of these preservation processes on sugar kelp were assessed

using physicochemical, sensory, and microbiological methods. To achieve this, two objectives were considered. The first objective of this study was to analyze the effect of blanching (100 °C for 1 or 3 minutes) on the physicochemical and microbial properties of sugar kelp and to conduct sensory evaluation of a food product (seaweed salad) developed from the blanched kelp, as compared to raw. This was done to determine the effect of minimal processing (blanching) on kelp quality and its impact on consumers' acceptance. The second study focused on the effects of blanching and freezing on fermented kelp products to offer interesting possibilities for development of other types of kelp foods. Our prior research found no significant differences in consumer liking of sugar kelp sauerkraut-style products made with raw kelp plus 25% or 50% cabbage (Skonberg et al. 2021). Because of the similarity of fermented kelp to sauerkraut, it will be referred to as "kelp- or kelp/cabbage sauerkraut" in this paper. The consumer liking of kelp sauerkraut formulated with blanched and/or previously frozen product is unknown. Therefore, the second objective of this study was to evaluate the effects of blanching and freezing of sugar kelp on the microbial quality, physical properties, and consumer acceptability of sauerkraut containing sugar kelp. A 50% kelp/cabbage sauerkraut blend was chosen for this study and was compared to a lab-made 100% cabbage sauerkraut. Findings are of economic significance to the seaweed industry as growers and processors attempt to diversify products and increase profit.

3.2 Materials and methods

3.2.1 Sample preparation

Fresh sugar kelp (*Saccharina latissima*) was received on two different occasions in a space of three weeks in April 2019 for the two experiments (kelp salad and sauerkraut studies). About ~95 kg of fresh, cultivated sugar kelp were harvested and received in coolers on ice from Maine Sea Farms (South Bristol, ME), approximately 30 kg and 65 kg for the kelp salad and sauerkraut study. Kelp samples were washed with tap water to remove debris and shredded with a food processor (RobotCoupe®, CL 50 Series E, Jackson, MS, USA) fitted with a 0.32 cm slicing disc. In both experiments, about 350 g of shredded kelp were weighed into 30.48 cm × 30.48 cm plastic bags (UltraSource, Kansas, MO, USA) and vacuum sealed under 99% vacuum (KOCH Ultravac, Model UV550, Wichita, KS, USA). Vacuum-packed bags of kelp were placed in a metal strainer and submerged in boiling tap water (100 °C) of about $\frac{3}{4}$ of a 50 L steam jacketed kettle for a prescribed time according to the experimental design. Internal temperature was not monitored during blanching. After blanching, the sample bags were immediately cooled in an ice/water slurry (~1 °C) for 1 min.

3.2.2 Kelp salad study

Kelp was separated into three groups: a 1-min blanched, 3-min blanched, and unblanched (control) treatments. Blanching temperature, blanching time and vacuum packaging were based on the relatively higher product quality recommended by a previous study in our laboratory (Akomea-Frempong et al. 2021a). Random samples were aseptically taken from the vacuumed bags after blanching and analyzed in triplicate for physicochemical and microbial quality (Figure 3.1). The remaining replicates of each

treatment were mixed together separately to prepare kelp salad. A seaweed salad recipe from Food.com (Food.com 2019) was modified for this purpose. The samples were then processed into a seaweed salad for sensory evaluation. Shredded kelp from the three previously processed treatments were mixed with shredded carrots (1.3% salad weight) and sesame seeds (10.1%), before adding 0.15% of commercial Asian balsamic vinaigrette (containing balsamic vinegar, vegetable oil (soybean and/or canola), extra virgin olive oil, salt, garlic, spice, onion, xanthan gum, red bell pepper, mustard flour) [Ken's Lite Balsamic Vinaigrette, MA, USA]. Three salad treatments (blanched for 1 min or 3 min, raw) were prepared to evaluate the effects of blanching treatment on the consumer acceptability of the kelp (Figure 3.1).

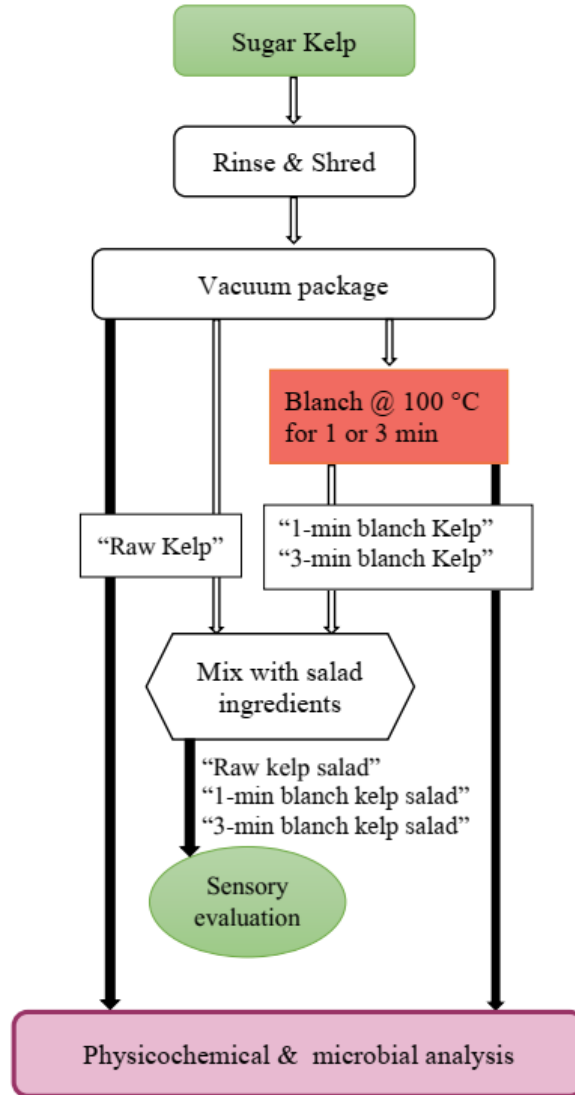


Figure 3.1: Flow diagram for kelp salad study

3.2.3 Kelp sauerkraut study

The kelp sauerkraut study was designed to test for the effect of blanching and freezing on physicochemical and microbial properties of sugar kelp, which was developed into a value-added food product (kelp sauerkraut). The shredded kelp was divided into four treatments: raw, raw/frozen ($-20\text{ }^{\circ}\text{C}$, 24 hr), blanched ($100\text{ }^{\circ}\text{C}$, 1 min), or blanched/frozen. Specifically, one of the blanched treatments (blanched/frozen) was immediately blast frozen after blanching, together with one of the raw kelp treatments

(raw/frozen) at -30 °C (Southeast Cooler, Lithia Springs, GA) for an hour, and then stored at -20 °C for 24 hours before further processing. White cabbage (*Brassica oleracea*) was purchased from a local grocer. The outer leaves of cabbage were discarded, and the rest were washed and shredded with the same food processor used for shredding kelp. The four kelp treatments were combined with shredded cabbage (50% ratio) and manually mixed with kosher salt (2% of kelp/cabbage mix weight, Morton coarse Kosher salt, Chicago, IL) for 5 min to produce a brine solution (Figure 3.2). The last treatment was 100% cabbage with 2% kosher salt, which served as a control. Each of the five treatments was packed into 3.785 L glass fermentation jars (Kombucha Brooklyn, Kingston, NY) with a plastic lid and airlock. Treatments were subsequently inoculated aseptically in triplicate with starter cultures (subheading 3.2.4) to ferment at ambient temperature (~22 °C) until a pH < 4.0 was achieved (an average of six days for all cabbage sauerkraut and nine days for kelp-containing sauerkrauts (Appendix C)). Kelp sauerkrauts were stored in a walk-in cooler at 4 °C for about 10 days prior to further analysis and sensory evaluation to simulate when a typical consumer might receive the commercial product after transport and stocking.

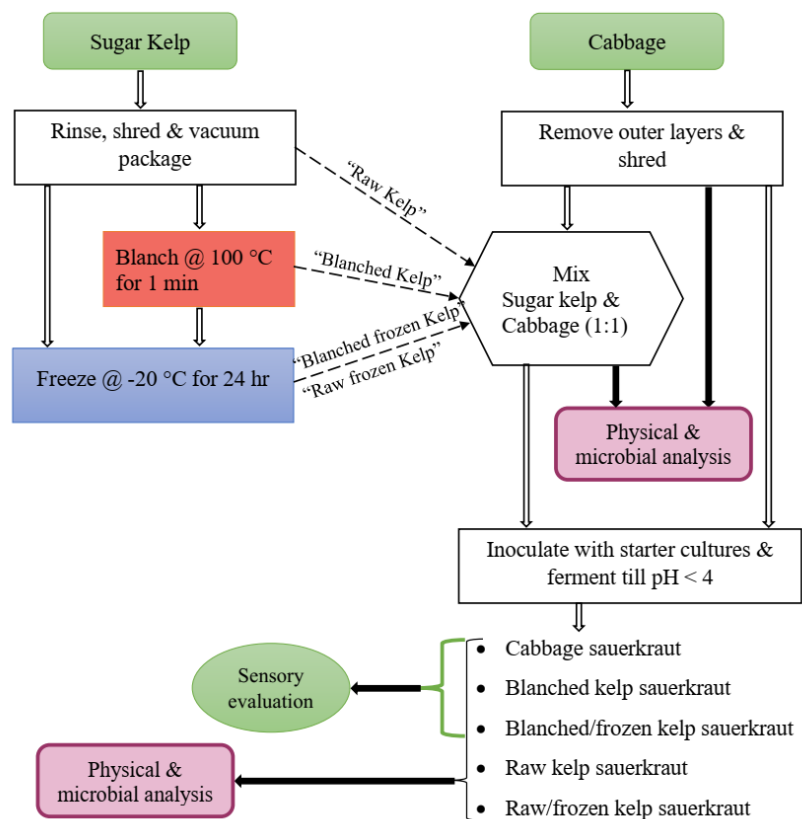


Figure 3.2: Flow diagram for kelp sauerkraut study

3.2.4 Starter culture preparation

Lactobacillus plantarum (ATCC 8014) and *Leuconostoc mesenteroides* subsp. *cremoris* were obtained from Microbiologics (St. Cloud, MN) and DuPont (Danisco, Paris, France), respectively. Cultures were stored at -80 °C before use. The cultures were streaked separately onto *Lactobacilli* MRS agar (Alpha Biosciences, Baltimore, MD) and placed into a 30 °C incubator for 48 hours. One single colony of each culture was aseptically transferred into 9 mL of room temperature *Lactobacilli* MRS broth (Alpha Biosciences, Baltimore, MD) and incubated at 30 °C for 24 hours to achieve a population of ~9 log CFU/g for both cultures, verified by direct plating, which was used to inoculate

the five treatments to achieve a target concentration of 10^1 CFU/g for *L. mesenteroides* and 10^6 CFU/g for *L. plantarum*.

3.2.5 Physicochemical analyses

3.2.5.1 Colorimetric analyses

Color change in sample treatments was measured with a colorimeter (LabScan XE, Hunter Labs, USA) fitted with a 5.1 cm diameter aperture, a port size of 5.05 cm, area view of 4.45 cm, and D65 illumination. The colorimeter was standardized with white and black tiles before each use and the colorimeter was allowed to warm up for 30 min prior to color analysis. Sample shreds were placed to cover the bottom of a transparent cup and Hunter L^* , a^* , b^* values were determined. Ten readings were recorded for each treatment replicate. Color change (ΔE) after processing was calculated in comparison to raw values using the following formula:

$$\Delta E_{ab}^* = \sqrt{(L_2^* - L_1^*)^2 + (a_2^* - a_1^*)^2 + (b_2^* - b_1^*)^2}$$

where L^* denotes lightness using a scale from black (0) to white (100), a^* denotes the red (+a) to green (-a) color axis, and b^* denotes the yellow (+b) to blue (-b) color axis. For the kelp salad study, the subscript 1 represents color values for raw samples before blanching and 2 represents color values after blanching.

3.2.5.2 Instrumental texture

Texture analysis for all treatments was conducted using the Kramer shear method with slight modifications (Johanningsmeier et al. 2007). Briefly, 10 – 15 g of shredded sample were loaded into a mini Kramer shear cell (TA-XTi2, Texture Technologies Inc, Scarsdale, NY, USA) with five flat blades set to travel 5 cm in a downward direction at a

pre-test and post-test speed of 2 mm/s. The texture analyzer was calibrated using a 5,000 g load cell before each use. The force (N) required to shear the sample was recorded by the texture analysis software (Exponent 32, version 5.0, 6.0, 2010, Texture Technologies Inc., Scarsdale, NY) as the firmness of the shredded kelp. Ten subsamples from each treatment replicate were analyzed, and values were averaged.

3.2.5.3 Moisture content

Moisture content (%) was determined using a convection oven (VWR International, Radnor, PA). Each treatment replicate was evaluated in duplicate, and values were averaged in percentage on a wet weight basis (wwb). Briefly, homogenized kelp samples (5 ± 0.002 g) in a pre-weighed aluminum pan were dried at 105 °C for 6 hours (AOAC, Method 950.46) (AOAC 2005a). Pans containing the dried samples were re-weighed and the percent moisture was calculated using the formula below:

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

3.2.5.4 Total phenolic content (TPC) and antioxidant analysis

Blanched and raw samples used for salad were freeze-dried (VirTis Ultra, Warminster, PA) using multiple 30h drying cycles until the samples reached a constant weight. The freeze-dried samples were ground using a coffee grinder (Hamilton Beach Fresh Coffee Grinder, USA), and stored at -80 °C until extracted for analysis as previously described by Rajauria et al. (2010) with slight modifications. Freeze-dried samples (2 g) were mixed with 20 mL of 60% methanol (v/v) and shaken on a lab plate shaker at 210 rpm for 24 h at room temperature. The mixture was centrifuged at $2100 \times g$ (Beckman Avanti J-25, Brea, CA) for 10 min. All supernatants from the extraction and

pellet wash (2 times) were collected and then brought to a final volume of 50 mL with deionized water. The extracts were stored at -20°C prior to conducting total phenolic content (TPC) and ferric reducing antioxidant power (FRAP) assays.

Total phenolics were determined in duplicate using the Folin-Ciocalteu reagent. Absorbance was measured at 725 nm against a 42% methanol blank. Total phenol content was expressed as mg of gallic acid equivalent (GAE) per g of freeze-dried sample based on a gallic acid reference curve (0-200 $\mu\text{g/mL}$) (Rajauria et al. 2010).

The assay for ferric reducing antioxidant power (FRAP) procedure was conducted according to the method described by Rajauria (Rajauria et al. 2010). FRAP reagents were prepared fresh daily. Fe^{3+} in the FRAP reagent, which included 2,4,6-tripyridyl-s-triazine (TPTZ), was reduced in the presence of the sample extracts, and a colored TPTZ- Fe^{2+} complex was formed. After 4 min, sample absorbance was measured at 595 nm against a deionized water sample blank. A standard curve was derived from the absorbances of 50-750 μM ferrous sulfate ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$) in deionized water. All samples were analyzed in duplicate and results were expressed as μmol ferrous sulfate equivalents (FSE) per gram of freeze-dried sample.

3.2.6 Determination of microbiological quality

In the kelp salad study, microbial safety analysis was performed on the raw control and blanched kelp treatments before incorporating them into salads. In the second study, samples were tested before and after fermentation of the five treatments. The presence of *Vibrio* spp., *Listeria monocytogenes*, *Salmonella* spp., and *Staphylococcus aureus* was assessed as described by FDA's Bacteriological Analytical Manual (FDA 2018c). Briefly, 25 g of each of the samples were placed aseptically into 225 mL of

alkaline peptone water (28 °C) for *Vibrio*, *Listeria* enrichment broth (28 °C) for *Listeria*, lactose broth (35 °C) for *Salmonella* and tryptic soy broth with 10% NaCl and 1% sodium pyruvate (35 °C) for *Staphylococcus aureus* in a stomacher bag and homogenized for two minutes using a BAGMixer 400 (Model P, Spiral Biotech, Advanced Instruments, Norwood, MA, USA). Afterward, the stomacher bag was incubated for 24 h and samples were plated (0.1 mL) on thiosulfate-citrate-bile salts-sucrose agar (28 °C, *Vibrio*), modified Oxford agar (28 °C, *Listeria*), xylose lysine deoxycholate agar (35 °C, *Salmonella*) and Baird-Parker (35 °C, *S. aureus*) in duplicate and incubated for 48 h for each of the treatment replicates. The presence of colony growth with expected morphology denoted the presumptive presence of pathogens.

To assess microbial quality, duplicate samples (10 g) of all treatment replicates in both experiments were mixed with 0.1% peptone and agitated for 2 min. After agitation, the samples were serially diluted in 0.1% peptone and spread plated onto tryptic soy agar (TSA) (Alpha Biosciences, Baltimore, MD) and acidified potato dextrose agar (APDA, Alpha Biosciences, Baltimore, MD) for aerobic plate counts (APC) and fungi, respectively. Plates were incubated at 37°C for 48 h (TSA), and at room temperature for 5 days (APDA). Microbial populations were determined in log CFU/g for APC and fungi.

3.2.7 Sensory evaluation

Consumer acceptability testing occurred at the University of Maine Sensory Evaluation Center (SEC) in Hitchner Hall on Wednesday, April 24th 2019 between the hours of 11:00 am and 5:00 pm for kelp salad and Wednesday, May 23rd 2019 between the hours of 11:00 am and 5:00 pm for kelp sauerkraut. This research was approved by the University of Maine Institutional Review Board for the protection of human subjects.

All research participants provided their informed consent (Appendix D). In the kelp salad study, sensory evaluation was conducted to determine the effects of two blanching times on consumer acceptance of salad made from blanched or raw kelp. Consumers tested three seaweed salads (1-min blanched, 3-min blanched, and unblanched (control) (Figure 3.3a). One hundred and two sensory panelists (at least 18 years old) in the greater Orono, ME area interested in seaweed and not allergic to seaweed or the other salad ingredients were recruited via email and flyer notices to assess the acceptability of sugar kelp salad (Appendix E). Each of the three salads was kept at 5 - 10 °C in a covered aluminum dish before being served. Panelists were simultaneously presented with three 30 g samples of three kelp salads for evaluation.

In the kelp sauerkraut study, 30 g of sauerkraut prepared as described previously was served for each of the three treatments: blanched kelp sauerkraut, blanched/frozen kelp sauerkraut, and the raw cabbage sauerkraut control (Figure 3.3b). Eighty sensory panelists (older than 18 years) interested in consuming seaweed and sauerkraut were recruited via email and flyer notices to assess the acceptability of kelp and/or cabbage sauerkraut (Appendix F). Each treatment was kept at 5 - 10 °C in a covered aluminum dish prior to being served.



Figure 3.3: a) Sugar kelp salad; b) Sugar kelp and/or cabbage sauerkraut

For both studies, panelists were seated in individual booths with a combination of fluorescent and incandescent lighting at the Sensory Evaluation Center at the University of Maine. During testing, the rooms were well-lit to control variables and biases. Distractions were kept to a minimum and differences were minimized among samples by filling each ramekin to about two thirds full (~30 g) of seaweed salad or sauerkraut. All samples were kept at a similar temperature by holding them in the refrigerator until serving. The three products were labeled with 3-digit random codes and were served in a ceramic ramekin with small cups of ~4 °C Poland spring water alongside. Sample order was randomized according to the SIMs software in each study to reduce the effects of flavor carry-over and order bias. Panelists were instructed to evaluate the samples, take a sip of water before testing each sample, and rate the acceptance of specific sensory attributes of the samples (Appendix G). A 9-point hedonic scale (from 1 = “Dislike Extremely” to 9 = “Like Extremely,” with 5 = “Neither Like nor Dislike”) was used to assess the acceptability of appearance, color, flavor, texture, and overall liking of samples (Peryam and Pilgrim 1957) and a 5-point Just-About-Right (JAR) scale (1 = Not Firm/Tender, 2 = Somewhat Firm/Tender, 3 = Just About Right, 4 = Somewhat Too Firm/Tender, and 5 = Much Too Firm/Tender) was used to examine specific texture attributes (firmness and tenderness) for salad only (Rothman and Parker 2009). Penalty analysis was performed for scores that were not JAR. Participants were asked to answer a set of questions relating to demographic characteristics, seaweed consumption habits, and attitudes towards consuming seaweed in both studies prior to consuming samples. Panelists were also asked if they would like to consume raw seaweed in the kelp salad study prior to consuming samples. Panelist were asked to select one descriptor that best

described each salad treatment from a short list (chewy, firm, tender, juicy, mushy, soft, tough) based on previous researches (Bell et al. 2017; Nayyar and Skonberg 2019). Also, panelists choose which forms they consume seaweed (as part of other foods like sushi, salad, soup, frozen smoothie cubes or in other form). In the kelp sauerkraut study, participants were additionally asked to check all that apply (CATA) for words that best described each sauerkraut sample after consumption. Panelists were asked to provide comments about the three treatments at the end of both studies. The test randomizations, experimental designs, and analyses were executed using SIMS 2000 (Sensory Computer Systems, Berkeley Heights, NJ, USA) software.

3.2.8 Statistical analysis

Data from physicochemical, microbial, and sensory tests were analyzed using SPSS 20 (IBM, Armonk, NY, USA) at a significance level of $P \leq 0.05$. One-way analysis of variance (ANOVA) was used to assess all one-level (treatment) effects. Multiway ANOVA was used to assess salad type and consumption frequency. Separation of treatment means was accomplished using Tukey's honest significant difference (HSD) post hoc test. Pearson's correlation was performed to evaluate correlations among variables. An independent t-test was used to compare the changes in color between the two blanched treatments in study one, and a pairwise t-test was used to compare kelp/cabbage qualities in treatments before and after fermentation in study two. A Cochran-Mantel-Haenszel test was used to determine whether JAR score distributions were different among the three products for firmness and tenderness attributes.

3.3 Results and discussion

Additional results and discussion that were not included in the published paper are presented in Appendix I.

3.3.1 Color

For the kelp salad study, blanching treatments significantly affected ($P \leq 0.05$) the color of sugar kelp irrespective of the blanching time (Table 3.1). The L^* and b^* values increased while the a^* values decreased when blanched. The difference in color between the raw kelp (control) and blanched kelp (ΔE value) was visible as a change from golden brown to a vivid bright green color.

Table 3.1: Color (Hunter L^* , a^* , b^*) of raw and blanched treatments of sugar kelp for salad [mean \pm SD (n = 3)]

Treatments	L^*	a^*	b^*	ΔE value	Firmness (N)
Raw	15.3 ± 1.7^c	3.9 ± 0.9^c	13.8 ± 1.5^b	--	280.2 ± 37.8^a
Blanched for 1 min	19.2 ± 2.8^b	-2.2 ± 0.9^a	18.5 ± 2.1^a	9.0 ± 1.8^a	227.1 ± 57.4^b
Blanched for 3 min	20.5 ± 1.5^a	-1.0 ± 1.0^b	17.8 ± 2.2^a	8.3 ± 1.7^a	182.3 ± 32.1^c

One-way ANOVA except for ΔE values (independent t-test).

Superscripts: different letters within column indicate significant differences among treatments ($P \leq 0.05$).

Hunter (L^* , a^* , b^*): L^* = lightness, a^* = red/green, b^* =yellow/blue, ΔE =Change in color.

Color is an important index for the quality of processed sugar kelp. The golden brown color of kelp immediately transformed to a green color when blanched, similar to the color change of kelp when blanched in other studies (Blikra et al. 2019; Bruhn et al. 2019). The high intensity of greenness seen in blanched kelp indicates a breakdown of the brown pigment fucoxanthin (Zhao et al. 2019), which masks the green color of chlorophyll in raw kelp. The longer blanching time (3 min) at 100 °C resulted in a lower green intensity as compared to the shorter blanching time (1 min). A longer exposure to heat likely led to the formation of chlorophyll breakdown products including the

brownish pigment pheophytin and the yellow brown olive pigment pyropheophytin, as a result of the replacement of the central magnesium atom with a hydrogen atom (Schwartz et al. 1981; Chen and Roca 2018). The trend was similar to the green color, expressed as a^*/b^* , of blanched winged kelp (*Alaria esculenta*) but contrary to that of sugar kelp samples, when they were subjected to various blanching temperatures (60 – 95 °C) and times (1 s – 60 min). Sugar kelp showed an upward trend of green color intensity (Blikra et al. 2019). Hunter a^* value had a mildly inverse correlation ($P \leq 0.0001$, $r = -0.389$) with the overall liking hedonic score of kelp salad, with inverse of a^* indicating the intensity of kelp greenness. These results highlight the need for strict control of blanching procedures to maximize consumer acceptability.

Regarding the kelp sauerkraut study, blanching and freezing of the kelp had no significant effects on a^* and b^* values of the four kelp/cabbage mix treatments prior to fermentation into kelp sauerkraut. Similarly, blanched sauerkraut treatments had no significant effect on a^* and b^* values as compared to raw treatments after fermentation. Kelp blanching resulted in significantly higher L^* values in blanched kelp/cabbage mix as compared to raw/frozen kelp/cabbage mix prior to fermentation, but this difference was no longer observable after completion of fermentation (Table 3.2a). Also, freezing was associated with decreased L^* values among raw treatments after fermentation (Table 3.2a). L^* , a^* , and b^* values for kelp sauerkraut (Table 3.2a) were similar to those of 50% sugar kelp sauerkraut-style product reported in the literature (Skonberg et al. 2021). There were no significant differences between the raw and blanched kelp/cabbage mix for a^* and b^* values, possibly due to the mixture of the white cabbage. Similarly, there was no significant change in color for b^* values (indicating yellowness) between raw

kelp sauerkraut and blanched kelp sauerkraut. A previous study also reported no change in the visual appearance descriptor (yellow-green) between fresh kelp and fermented kelp when subjected to a descriptive sensory test by 13 panelists (Bruhn et al. 2019).

3.3.2 Instrumental texture

The textural parameter determined in kelp samples was shear force (Firmness, N). Blanching decreased kelp firmness, especially when blanching time increased from 1 to 3 minutes (Table 3.1). Kelp firmness decreased as blanching time progressed, suggesting a thermal breakdown of polysaccharides in kelp cell walls. Kelp polysaccharides are comprised mainly of alginate that consists of unbranched chains of contiguous β -1,4-linked D-mannuronic acid blocks, and blocks of contiguous α -1,4-linked L-guluronic acid (Percival 1979; Graham et al. 2016), which become porous when heated. The increase in moisture content after blanching may have been due to the abundant kelp polysaccharides absorbing and retaining some of the water molecules which would have been lost to dripping in a raw product (Serp et al. 2002; Rezende et al. 2007; Wang et al. 2013). There is a possibility that the increase in moisture content may result in increased profits for kelp processors since finished products are sold by weight.

Kelp was blanched and/or frozen before mixing with cabbage prior to fermentation. For the kelp/cabbage mix prior to fermentation, blanching significantly decreased ($P = 0.00$, F-statistic = 152.86) firmness in both blanched, as compared to raw, treatments but freezing significantly decreased firmness in only raw treatments (Table 3.2b).

Table 3.2a: Color (Hunter L*, a*, b*) of kelp and/or cabbage mix treatments before and after fermentation [mean \pm SD (n = 3)]

Treatments <i>fermentation</i>	L*		a*		b*	
	Before	After	Before	After	Before	After
Cabbage only	67.2 \pm 2.1 ^{aA}	65.1 \pm 1.0 ^{aA}	0.6 \pm 0.9 ^{aA}	0.3 \pm 0.7 ^{cA}	27.6 \pm 2.7 ^{aA}	27.0 \pm 1.2 ^{aA}
Raw kelp and cabbage	40.2 \pm 2.7 ^{bcA}	42.6 \pm 1.2 ^{bA}	2.1 \pm 1.1 ^{aA}	2.1 \pm 0.4 ^{aA}	16.0 \pm 1.4 ^{bA}	16.9 \pm 1.1 ^{bA}
Raw/frozen kelp and cabbage	40.0 \pm 1.6 ^{cA}	38.7 \pm 1.0 ^{cA}	2.1 \pm 1.2 ^{aA}	2.1 \pm 0.9 ^{aA}	14.7 \pm 1.0 ^{bA}	15.7 \pm 0.7 ^{bA}
Blanched kelp and cabbage	43.6 \pm 1.9 ^{bA}	40.6 \pm 1.3 ^{bcA}	2.0 \pm 1.0 ^{aA}	1.6 \pm 0.8 ^{abcA}	14.9 \pm 0.8 ^{bA}	15.8 \pm 0.9 ^{bA}
Blanched/frozen kelp and cabbage	44.0 \pm 2.2 ^{bA}	40.4 \pm 1.1 ^{bcB}	1.8 \pm 1.6 ^{aA}	1.8 \pm 0.9 ^{abA}	15.2 \pm 1.0 ^{bA}	16.5 \pm 1.0 ^{bA}

Before fermentation samples were 50% kelp/cabbage mixture and samples were 50% kelp/cabbage sauerkraut after fermentation.

One-way ANOVA among treatment (column); pairwise t-test before and after fermentation (row).

Superscripts: different small letters indicate significant differences among treatments (within column); different capital letters indicate a significant difference before and after fermentation (within row). A probability level of 0.05 ($P \leq 0.05$) was selected for significance.

Hunter (L*, a*, b*): L* = lightness, a*= red/green, b*=yellow/blue.

Table 3.2b: Texture of kelp and/or cabbage mix treatments before and after fermentation [mean \pm SD (n = 3)]

Treatments <i>fermentation</i>	Firmness (N)	
	Before	After
Cabbage only	274.4 \pm 10.6 ^{aB}	233.9 \pm 15.1 ^{aA}
Raw kelp and cabbage	238.4 \pm 14.2 ^{bA}	225.4 \pm 15.0 ^{aA}
Raw/frozen kelp and cabbage	229.5 \pm 16.1 ^{bcA}	225.7 \pm 15.1 ^{aA}
Blanched kelp and cabbage	201.0 \pm 12.3 ^{cA}	188.5 \pm 13.7 ^{bA}
Blanched/frozen kelp and cabbage	199.4 \pm 14.5 ^{cA}	198.1 \pm 11.3 ^{bA}

Before fermentation samples were 50% kelp/cabbage mixture and samples were 50% kelp/cabbage sauerkraut after fermentation.

One-way ANOVA among treatment (column); pairwise t-test before and after fermentation (row).

Superscripts: different small letters indicate significant differences among treatments (within column); different capital letters indicate a significant difference before and after fermentation (within row). A probability level of 0.05 ($P \leq 0.05$) was selected for significance.

After fermentation, freezing had no impact on kelp sauerkraut treatments but blanching significantly reduced ($P = 0.00$, F-statistic = 115.94) firmness in kelp sauerkraut as compared to raw treatments. When comparing the firmness of each treatment pre- and post-fermentation, only the 100% cabbage control significantly decreased (Table 3.2b).

The range of firmness values for kelp/cabbage sauerkraut in our study (Table 3.2b) was higher than for fermented kelp/cabbage sauerkraut stored at 3 °C for 60 days post inoculation (< 150 N) (Skonberg et al. 2021). This indicates that sauerkraut firmness may have decreased as fermentation progressed during low-temperature storage. When comparing products prepared from blanched fresh vs. blanched/frozen kelp, freezing did not have a significant immediate effect on the color or firmness of the kelp sauerkraut. Thus, freezing may provide seaweed producers with an alternative to prolong the shelf-life of sugar kelp for subsequent food production. Similarly, the firmness of frozen blanched sugar kelp remained unchanged during six months of frozen storage in a previous study conducted in our laboratory (Akomea-Frempong et al. 2021a). It would be valuable to see whether longer-term frozen storage of the kelp (e.g. 1 year) would impact subsequently prepared sauerkraut texture.

3.3.3 Chemical properties

Blanching had a significant impact on moisture content, which ranged from 86.3 to 91.5% (wwb). The longer blanching time resulted in significantly higher moisture content as compared to raw kelp (Table 3.3). No significant trends in TPC and FRAP values were observed based on the blanching time (Table 3.3).

Table 3.3: Chemical properties of raw and blanched treatments of sugar kelp for salad [mean \pm SD (n = 3)]

Treatment	Moisture (%)	TPC (mg GAE/g)	FRAP (μ mol FSE/g)
Raw	86.3 \pm 5.0 ^b	1.5 \pm 0.7 ^a	5.3 \pm 1.6 ^a
Blanched for 1 min	90.6 \pm 0.8 ^{ab}	1.1 \pm 0.6 ^a	3.6 \pm 0.8 ^a
Blanched for 3 min	91.5 \pm 0.4 ^a	0.8 \pm 0.3 ^a	3.9 \pm 2.0 ^a

TPC = Total phenolic content. FRAP = ferric reducing antioxidant power.

TPC and FRAP are measured in gram of freeze-dried sample.

Superscripts: different letters within column indicate a significant difference among treatments ($P \leq 0.05$).

Blanching slightly decreased total phenolic contents (TPC), and antioxidant capacity as determined by the FRAP method. The observed low values of TPC and FRAP in all kelp salad treatments may be as a result of shredding as seen in our previous shredded frozen kelp study (Akomea-Frempong et al. 2021a). Although no significant differences in TPC or FRAP values were found among treatments, the slight decline in TPC and FRAP values as blanching time increased suggests a negative impact of thermal treatment in preserving phenolic compounds and antioxidant capacity in sugar kelp, as expected. TPC values in the present study for fresh and blanched kelp treatments (Table 3.3) are below the range for fresh and blanched sugar kelp (2.4–54.4 mg·GAE/g (Nielsen et al. 2020)) and within the range of fresh harvested sugar kelp in different seasons (0.84–2.41 mg·GAE/g (Marinho et al. 2019)) reported in different studies. FRAP values were within the range of total antioxidant capacity (TAC) in fresh harvested sugar kelp in different seasons (0.84–2.41 mg·GAE/g DM (Marinho et al. 2019)).

Overall, blanching may aid in commercializing kelp products because it increased the moisture, lightness, and greenness of kelp, which positively impacted sensory scores. The optimal texture preferences of consumers should be defined in future research.

3.3.4 Microbiological quality

Considering the kelp salad study, raw samples were compared to blanched samples with emphasis on the effects of blanching time on microbial quality. There were no significant differences in APC or fungi counts among raw, 1 min and 3 min blanching time samples, which were below 3 log CFU/g and 2.5 log CFU/g, respectively (Table 3.4). None of the pathogens tested (*Vibrio* spp., *Listeria monocytogenes*, *Salmonella* spp., and *Staphylococcus aureus*) were detected in any of the samples.

In the kelp sauerkraut study, APC and fungi counts before fermentation ranged from 2.0 – 2.4 log CFU/g (Table 3.4). Blanching and freezing had no impact on APC or fungi counts. When comparing the APC and fungi counts in the different treatments before and after fermentation, only raw kelp sauerkraut had a significant increase in the fungi population after fermentation. While not measured in this study, previous work (Skonberg et al. 2021) has shown that levels of lactic acid bacteria are closely negatively correlated with pH, and so are expected to have increased proportionally during fermentation. A presumptive positive result for *Vibrio* sp. was detected in one replicate of the raw kelp/cabbage mix samples but was not detected after fermentation.

Aerobic plate count (APC) and fungi counts were low in both experiments, suggesting a minimal risk of kelp salad and kelp sauerkraut spoilage from microorganisms. The results were similar to previously reported microbial populations (between 1 and 3 log CFU/g) of *Alaria esculenta* and *Saccharina latissima* when subjected to different heat treatments (Blikra et al. 2019). Blanching significantly reduces microflora in vegetables, where either below or near the detection level (1 log CFU/g)

reduction was observed in *Enterobacteriaceae*, total yeast, and mold counts (Edgar and Aidoo 2001).

Table 3.4: Enumeration of aerobic plate count and fungi of sugar kelp in the two experiments [mean \pm SD (n = 3)]

Treatment	APC (Log CFU/g)		Fungi (Log CFU/g)	
<i>Salad study</i>				
Raw	2.9 ± 0.4 ^a		2.1 ± 0.3 ^a	
Blanched for 1 min	2.6 ± 0.2 ^a		2.4 ± 0.5 ^a	
Blanched for 3 min	2.4 ± 0.5 ^a		2.2 ± 0.4 ^a	
<i>Sauerkraut study</i>				
(fermentation)	Before	After	Before	After
Cabbage only	2.2 ± 1.0 ^{aA}	2.2 ± 0.8 ^{aA}	2.3 ± 1.7 ^{aA}	2.2 ± 0.1 ^{aA}
Raw kelp/cabbage	2.3 ± 1.1 ^{aA}	2.1 ± 0.7 ^{aA}	2.2 ± 1.3 ^{aB}	2.5 ± 0.3 ^{aA}
Raw frozen kelp/cabbage	2.3 ± 0.9 ^{aA}	2.4 ± 0.5 ^{aA}	2.0 ± 0.9 ^{aA}	2.0 ± 0.2 ^{aA}
Blanched kelp/cabbage	2.3 ± 0.6 ^{aA}	2.2 ± 0.1 ^{aA}	2.0 ± 0.8 ^{aA}	2.1 ± 0.1 ^{aA}
Blanched frozen kelp/cabbage	2.4 ± 1.0 ^{aA}	2.1 ± 0.4 ^{aA}	2.4 ± 0.1 ^{aA}	2.4 ± 0.2 ^{aA}

APC = Aerobic plate count. Before fermentation samples were 50% kelp/cabbage mixture and samples were 50% kelp/cabbage sauerkraut after fermentation.

One-way ANOVA among treatment; pairwise t-test before and after fermentation

Superscripts: different small letters indicate significant difference among treatments; different capital letters indicate significant difference before and after fermentation ($P \leq 0.05$).

A similar reduction in APC and fungi counts of kelp was observed in both experiments after blanching; however, the reductions were not significant. For sauerkraut, Khanna (2019) reported similar fungi count range (~ 2.5 log CFU/g) and higher APC range (3.9-4.6 log CFU/g) in cabbage sauerkraut as compared to our study. About 8 log CFU/g of APC was observed in another cabbage sauerkraut study after two days of fermentation, which had a slight but not significant reduction in APC as fermentation progressed for 37 days (Wolkers-Rooijackers et al. 2013). Because initial levels of APC in this study were extremely low, it is not surprising that a significant decrease attributable to fermentation was not observed. When cabbage was mixed with kelp, about a 23% increase in APC was observed when different ratios of kelp/cabbage

mixture were fermented into sauerkraut in a different study, and levels of lactic acid bacteria were negatively correlated with pH (Skonberg et al. 2021). The impact of fermentation on microflora (APC) of cabbage and/or kelp sauerkraut in the kelp sauerkraut study was not significant except in one treatment (Table 3.4).

Based on the numerous microbial pathogens and toxins found in the marine environment that are linked to human diseases (Thompson et al. 2005) and potential cross contamination during post-harvest processing of seaweed (Gupta et al. 2010), there is a possibility of harborage of pathogens on sugar kelp during production and processing. Water temperatures in the marine environment where seaweed is grown are increasing and these high temperatures are associated with elevations of *Vibrio* populations (Turner et al. 2009). Besides, there have been outbreaks of salmonellosis, listeriosis and *Staphylococcus aureus* poisoning associated with minimally processed or ready to eat vegetables via contaminations (Quiroz-Santiago et al. 2009; Zhu et al. 2017; Wu et al. 2018). Therefore, seaweed could be contaminated if not handled properly. The presence of *Staphylococcus aureus*, *Salmonella*, *Listeria monocytogenes*, and *Vibrio* was assessed in all treatments to ensure food safety. However, the absence of these pathogens in the kelp salad study is encouraging for the marketability of fresh kelp. The detection of a presumptive *Vibrio* colony in one replicate of the raw (fresh) kelp/cabbage mix (before fermentation) sample suggests that the presence of *Vibrio* sp. on kelp should be expected to be sporadic since *Vibrio* sp. are common in the waters where kelp is grown. Interestingly, all samples of fully fermented sauerkrauts were negative for presumptive *Vibrio*. Results reinforce the knowledge that fermentation conditions, especially the decrease in pH, can inactivate pathogens in some fermented food products. Similarly,

Bacillus cereus was absent in inoculated kelp after heat treatment and fermentation (Bruhn et al. 2019) and there was a reduction of pathogen growth as pH declined when cabbage was fermented with *Lactobacillus plantarum* (Lee and Lee 2010) and *Leuconostoc mesenteroides* (Choi et al. 2003). Moreover, several studies have reported the antimicrobial activity of seaweed, which is higher in brown seaweed extracts than red or green (Edgar and Aidoo 2001; Cox et al. 2010). Exudates from kelp as a result of shredding may have released bacteriostatic compounds from this brown seaweed which could act against spoilage microorganisms and pathogens. However, an inoculation study is recommended to confirm whether the fermentation process can inactivate pathogens present in the kelp/cabbage products.

3.3.5 Sensory evaluation

3.3.5.1 Demographics and consumption trends

Demographic and consumption habit questions were asked before the evaluation of the salads. More females (64%) took part in the evaluation (Table 3.5). The majority (72.5%) of the sensory participants for the kelp salad evaluation were 35 years old or younger. Sixteen participants were Asian, and 78 were Caucasian.

Table 3.5: Demographics of participants for kelp salad and sauerkraut sensory evaluation

Parameters		Salad study n = 102 (%)	Sauerkraut study n = 80 (%)
Gender	M	36 (35.3)	32 (40.0)
	F	65 (63.7)	48 (60.0)
	Did not answer	1 (1.0)	-
Age (years)	18-25 years	43 (42.2)	17 (21.2)
	26-35	31 (30.4)	39 (48.7)
	35-45	10 (9.8)	11 (13.8)
	46-55	7 (6.9)	5 (6.3)
	56+	11 (10.7)	8 (10.0)
Race	American Indian/Alaska Native	1 (0.9)	0 (0.0)
	Asian	16 (15.7)	23 (28.8)
	Black/African American	5 (5.0)	4 (5.0)
	White (Caucasian)	78 (76.5)	50 (62.5)
	Prefer not to say	0 (0.0)	3 (3.7)
	Did not answer	2 (1.9)	-

The participants indicated that seaweed was consumed more at restaurants than at home. Results showed that 64.7% of participants eat seaweed raw, 74.5% of participants consume it as part of other food like sushi, 44.1% as salad, 35.3% as soup, and the remainder in other forms, including frozen kelp smoothie cubes. More than half of the panelists (61.8%) chose flavor as the most important seaweed characteristic and color as the least (<1%). Also, 87.2% of participants indicated a willingness to buy a 113.4 g (4 oz.) bowl of seaweed salad for a \$ 2 – \$ 4 price range (Table 3.6).

Sixty percent of the participants in the kelp sauerkraut study were female and 70% of participants were younger than 35 years of age (Table 3.5). More than half of the participants were Caucasian (~63%) and about 29% were Asian. About 41% of participants claimed to consume seaweed 1-6 times a year, and 30% reported consuming 1-2 times a month. Over 75% of participants knew that fermented foods, such as

sauerkraut, may contain probiotics that are associated with disease prevention and improved digestion; 48.8% of panelists reported consuming probiotics as either a food or dietary supplement ≥ 1 time per week (Table 3.6).

Table 3.6: Responses of consumption behavior of participants for kelp salad and sauerkraut sensory evaluation

Parameters		Salad study n = 102 (%)	Sauerkraut study n = 80 (%)
Would you like to consume your seaweed raw?	Yes	66 (64.7)	N/A
	No	32 (35.3)	
Where do you usually consume seaweed?	Restaurant	58 (56.9)	N/A
	Home	24 (23.5)	
	Other	8 (7.8)	
	Not applicable	9 (8.8)	
	Did not answer	3 (2.9)	
Approximately how often do you consume seaweed?	< 1 year	9 (8.8)	34 (42.9)
	1-2 times a year	34 (33.3)	N/A
	1-6 times/year	N/A	32 (40.0)
	1-2 times a month	17 (16.8)	11 (13.8)
	2-3 times a month	34 (33.3)	N/A
	Weekly	6 (5.9)	N/A
	> 2 times a week	2 (1.9)	N/A
	Weekly or > 1 time a week	N/A	3 (3.7)
What would make you consume seaweed more often? (CATA)	Availability	72 (70.6)	N/A
	Ready-to-eat	53 (51.9)	
	Lower price	34 (33.3)	
	Sustainability	34 (33.3)	
	Sold fresh	31 (30.4)	
	Minimally processed	26 (25.5)	
	Longer shelf-life	21 (20.6)	
What form of seaweed products do you typically consume?	As part of other foods like sushi	76 (74.5)	N/A
	Salad	45 (44.1)	
	Soup	36 (35.3)	
	Frozen smoothie cubes	2 (1.9)	
	Other forms	16 (15.7)	

Table 3.6 continued

Price for a ready-to-eat four-ounce (113.4 g) seaweed salad bowl?	Would not buy	8 (7.8)	
	\$ 2.00	24 (23.5)	
	\$ 3.00	41 (40.2)	N/A
	\$ 4.00	24 (23.5)	
	\$ 5.00	5 (5.0)	
Which sensory characteristic of seaweed is most important to you?	Aroma	6 (5.9)	
	Color	3 (2.9)	N/A
	Flavor	63 (61.8)	
	Texture	30 (29.4)	
Did you know that fermented foods, such as sauerkraut, contain probiotics?	Yes		19 (23.8)
	No	N/A	61(76.2)
How often do you eat foods or dietary supplements containing probiotics?	Less than once per year		5 (6.3)
	1-4 times per year		15 (18.7)
	1-2 times per month	N/A	21 (26.3)
	1-2 times per week		23 (28.7)
	3+ times per week		16 (20.0)

3.3.5.2 Sensory attributes

The mean acceptability scores for five sensory attributes (appearance, color, flavor, texture, and overall liking) of the kelp salad ranged from 5.4 to 6.7 on the 9-point hedonic scale, which were between “neither like nor dislike” and “like moderately” (Table 3.7). Generally, the blanched samples used to prepare kelp salad were liked more than the raw sample for color, flavor, and overall liking (Table 3.7). No significant differences were seen in any sensory attributes between the blanched treatments. Overall acceptability scores for all three treatments had strong, significant ($P \leq 0.01$) positive correlations with texture ($r = 0.67$) and flavor sensory scores ($r = 0.91$). Notably, frequent (at least 2-3 times a month) consumers of seaweed and those that normally consume seaweed at restaurants rated the 3-min blanched kelp salad significantly higher than the 1-min and raw kelp salad for “overall liking.”

Table 3.7: Mean scores for consumer acceptance of raw and blanched kelp salad on a 9-point hedonic scale [mean \pm SD (n = 102)]

Attributes	Raw	1 min blanch	3 min blanch
Appearance	6.3 \pm 1.5 ^a	6.5 \pm 1.6 ^a	6.6 \pm 1.4 ^a
Color	6.1 \pm 1.7 ^b	6.5 \pm 1.4 ^a	6.5 \pm 1.4 ^{ab}
Texture	6.4 \pm 1.5 ^a	6.5 \pm 1.4 ^a	6.6 \pm 1.6 ^a
Flavor	5.5 \pm 1.9 ^b	6.5 \pm 1.7 ^a	6.6 \pm 1.7 ^a
Overall liking	5.7 \pm 1.7 ^b	6.5 \pm 1.7 ^a	6.5 \pm 1.7 ^a

Each value is the mean \pm standard deviation (n = 102).

Superscripts: different small letters within rows indicate significant difference among treatments ($P \leq 0.05$).
1 = Dislike Extremely and 9 = Like Extremely.

“Chewy” and “firm” were the CATA descriptors selected most frequently to describe the characteristics of the three kelp salad treatments (Table 3.8). Assessment of descriptors did not significantly differ ($P > 0.05$) when compared with the other treatments using chi-squared test.

Table 3.8: Descriptors selected for each kelp salad treatment (n = 102)

Descriptors	Raw kelp	1-min blanched kelp salad	3-min blanched kelp salad
Chewy	27	26	29
Firm	23	25	28
Tender	23	21	15
Juicy	7	15	10
Mushy	9	8	9
Soft	8	6	8
Tough	5	1	3

The subsequent JAR analysis focused on the specific texture attributes “firmness” and “tenderness,” and whether consumers considered them to be ideal. Results from JAR analysis among the salad treatments are shown in Figure 3.4. For an attribute to be considered ideal, at least 70% of the responses should be “Just About Right” (Rothman and Parker 2009). Above 20% of respondents judged all three salad products to be too firm and not tender, and none of the attributes had the right degree of firmness and

tenderness as JAR did not reach the ideal 70% mark (Figure 3.4). The Cochran-Mantel-Haenszel test showed no statistically significant differences among the three products in the distributions of the assessors' scores on the JAR scale for the firmness ($P > 0.05$; 0.698) and tenderness ($P > 0.05$; 0.776) attributes.

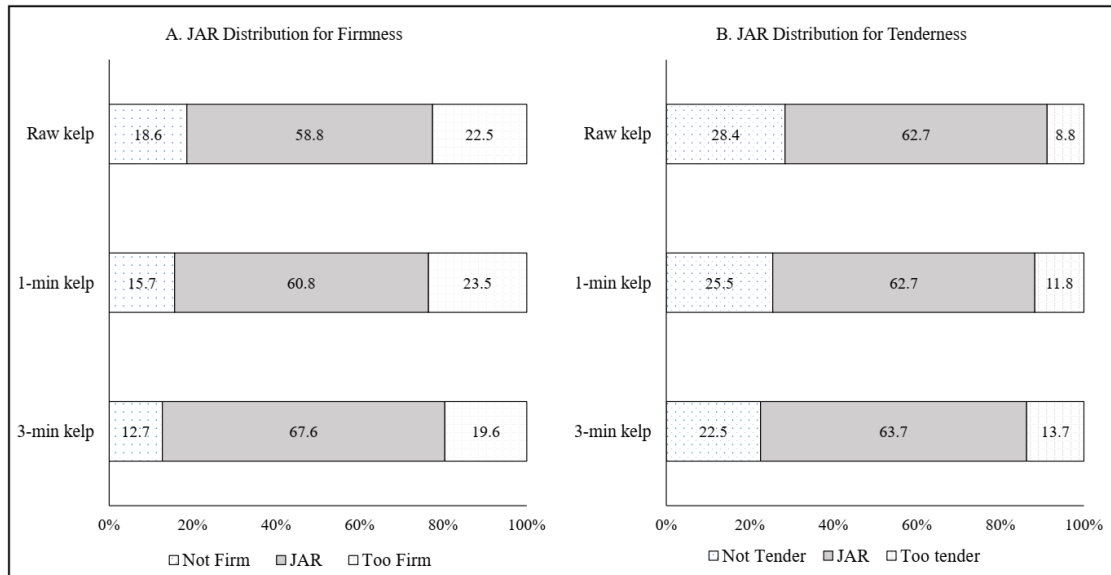


Figure 3.4: Just-About-Right (JAR) categorical scores ($n = 102$ consumers) for (A) firmness and (B) tenderness for raw kelp (control), 1-min blanched kelp, and 3-min blanched kelp salad

Penalty analyses of the raw kelp, 1-min blanched kelp, and 3-min blanched kelp salad samples were performed to determine whether respondents' ratings for firmness and tenderness which were not JAR (less than 70 % of responses were JAR) were associated with a mean drop in hedonic ratings of the Overall liking (Figure 3.5). Mean drops of 1.5 – 1.9 are concerning, drops of 1 – 1.49 are slightly concerning, and 0 – 0.99 are very slightly concerning (Peryam and Pilgrim 1957; Rothman and Parker 2009). Raw kelp and 3-min blanched kelp salad samples received concerning penalties for “Not enough tenderness,” while 1-min blanched kelp salad samples received concerning

penalties for “Too much firmness”. These mean drops reflected on the “overall liking” mean hedonic scores of raw kelp salad (5.7 ± 1.7), 1-min blanched kelp (6.5 ± 1.7), and 3-min blanched kelp salad samples (6.5 ± 1.7).



Figure 3.5: Mean drops (penalties) in overall liking on a 9-point hedonic scale (n=102 consumers) from penalty analysis corresponding to the scale ends for each JAR texture attribute of firmness and tenderness for raw kelp, 1-min blanch kelp, and 3-min blanch kelp salad samples

Generally, a mean liking score of ≥ 7 on a 9-point hedonic scale is associated with highly acceptable sensory quality (Everitt 2009). The overall liking scores for sensory evaluation for the salad treatments (raw, 5.7; 1-min blanched, 6.5 and 3-min blanched/frozen, 6.5) suggest that blanching had a positive impact on consumer acceptance of kelp. Since seaweed products are less popular in the West compared to Asian nations, it is important to note that the hedonic scores are promising because most of the panelists identified as Caucasian. The mean acceptability scores for color, texture,

flavor, and overall liking of seaweed for all the salad treatments fell within the range of 5.5 – 6.7, which is approximately within the 6-point score comparable to the “like slightly” category. The large variation in “overall liking” for raw kelp salad (5.7 ± 1.4), 1-min blanched (6.4 ± 1.7) and 3-min blanched/frozen (6.5 ± 1.7) may be a result of many respondents (42.2%) being infrequent seaweed consumers (< 1-2 times a year). A MANOVA analysis indicated the frequent consumption group (2-3 times a month to ≥ 1 in a week) rated the “overall liking” of raw, 1 min-, and 3 min blanched kelp salad as 5.7, 6.3, 7.2, respectively. Three-minute blanched kelp salad was rated significantly higher than raw kelp for overall liking, suggesting that blanching time influenced how respondents familiar with seaweed products liked kelp salad. The relatively higher ratings of blanched kelp compared to raw kelp salad samples (Table 3.9) may be due to the noticeably juicy and tender nature described by sensory participants. As previously noted, this texture could be a result of the increase in moisture content in blanched kelp. However, participants did not deem blanched treatments or raw kelp salads to be ideal for texture (chewiness and tenderness) from the JAR analysis, possibly as a result of the heterogeneity of kelp products. Consumers were able to differentiate between the color of the two blanching treatments and raw samples, which strongly correlated with instrumental color analysis. The greenness of kelp after blanching correlated to the overall liking of salad and it could be that green represented a more familiar vegetable product because of consumers’ perceptions about the color green and nature (Sliburyte and Skeryte 2014). In view of the high ratings for blanched kelp color, blanched products (kelp/cabbage sauerkraut) were selected as the focus for study two and they were compared to cabbage sauerkraut for sensory evaluation.

The mean acceptability scores for the control cabbage sauerkraut were higher for flavor and overall liking than for the blanched and blanched/frozen kelp sauerkrauts (Table 3.9). There were no differences among samples for appearance, color, and texture. The aroma of the blanched kelp sauerkraut had a lower mean hedonic rating than the sauerkraut with cabbage alone. Liking of blanched kelp sauerkraut was not significantly different from blanched/frozen sauerkraut for all sensory attributes. Overall acceptability scores for all sauerkraut treatments had strong, significant ($P \leq 0.01$) positive correlations with texture ($r = 0.63$), aroma ($r = 0.64$), and flavor scores ($r = 0.90$). Focusing on kelp sauerkraut only, overall acceptability scores had significant ($P \leq 0.01$) moderate positive correlation with texture ($r = 0.61$), and aroma (0.61); and strong, significant ($P \leq 0.01$) positive correlations with flavor scores ($r = 0.91$). The study showed no significant differences in “overall liking” scores between low (< 1 time a year) and high (≥ 1 time a month) frequency consumers of sauerkraut. High frequency consumers rated the blanched kelp sauerkraut (6.5) and blanched/frozen kelp sauerkraut (6.7) higher than the less frequent consumers of sauerkraut (both kelp treatments = 5.8).

Table 3.9: Mean scores for consumer acceptance of raw cabbage, blanched- and blanched/frozen- sauerkraut on a 9-point hedonic scale

Attributes	Sauerkraut		
	Raw cabbage	Blanched kelp	Blanched/frozen kelp
Appearance	6.7 ± 1.4^a	6.5 ± 1.6^a	6.3 ± 1.6^a
Color	6.5 ± 1.5^a	6.5 ± 1.5^a	6.3 ± 1.5^a
Aroma	6.3 ± 1.6^a	5.5 ± 1.8^b	5.7 ± 1.8^{ab}
Flavor	6.8 ± 1.4^a	5.9 ± 1.9^b	6.1 ± 1.8^b
Texture	7.0 ± 1.3^a	6.8 ± 1.4^a	6.7 ± 1.4^a
Overall liking	6.8 ± 1.4^a	6.0 ± 1.9^b	6.1 ± 1.7^b

Each value is the mean \pm standard deviation ($n = 80$).

Superscripts: different small letters within rows indicate significant difference among treatments ($P \leq 0.05$).

1 = Dislike Extremely and 9 = Like Extremely.

The majority of panelists described all sauerkraut treatments (raw cabbage-, blanched kelp- and blanched/frozen sauerkraut) as “crunchy,” and “pickled” (Table 3.10). Assessment of descriptors using chi-squared indicated significant differences ($P \leq 0.05$) among treatments. Cramer’s V coefficient (0.243) indicates that sauerkraut treatment had a small to medium effect on sauerkraut descriptors (Portney 2020). Interestingly, $\geq 25\%$ of panelists described all treatments as fresh and kelp sauerkraut as having ocean breeze flavor. Notably, panelists described blanched fresh kelp sauerkraut as “pungent” as compared to blanched/frozen sauerkraut, whereas as “well-rounded product” was used to describe blanched/frozen sauerkraut as compared to blanched fresh sauerkraut. A few panelists described the sauerkraut treatments in the comment section as “looks bright and smells good,” “color was more interesting in seaweed sauerkraut than cabbage only,” and “very acidic” (Appendix H).

Table 3.10: Descriptors selected for each sauerkraut treatment based on a check –all – that apply question (CATA)^a

Descriptors	Cabbage sauerkraut	Blanched fresh sauerkraut	Blanched/frozen sauerkraut
Crunchy	54	53	45
Pickled	54	46	42
Sour	42	38	31
Salty	37	56	50
Traditional kraut	35	5	9
Fresh	34	20	20
Tangy	31	30	27
Clean	17	13	9
Pungent	13	21	14
Boiled cabbage	12	8	8
Well rounded	10	6	14
Bland	7	0	0
Ocean breeze	6	24	23
Sweet	6	4	6

Table 3.10 continued

Mild	6	2	12
Bitter	5	11	10
Fizzy	4	3	2
Metallic	3	8	7
Mellow	3	2	4
Brackish	2	18	17
Fishy	2	24	22
Musty	2	2	4
Soggy	2	3	3
Slimy	2	8	9
Soft	1	5	5
Mushy	0	2	5

^a CATA = choose all that apply. Values shown are counts. Participants could check as many descriptors as they wished.

Scores from the sensory evaluation study of kelp sauerkraut suggest that fermentation could be used as an alternative method to produce seaweed foods for the consumer market. Although over three-quarters of the panelists knew fermented foods such as sauerkraut had probiotics, it did not correspond to a higher sauerkraut or seaweed consumption. Moreover, familiarity with probiotics in fermented foods did not significantly impact the sensory attribute “overall liking” among sauerkraut treatments (cabbage = 6.4 ± 1.7 , blanched kelp = 6.4 ± 1.5 , blanched/frozen kelp = 6.8 ± 1.6). Comments such as “looks bright and smells good” and “color was more interesting in seaweed sauerkraut than cabbage only,” among others, suggest that the bright colors of the sugar kelp mixed with cabbage were more appealing to some consumers than the pale color of cabbage only. However, no significant differences were recorded among treatments based on the hedonic color score means. Texture was the most highly rated attribute of all the sauerkraut treatments compared to a previous seaweed sauerkraut study (Skonberg et al. 2021). The majority of respondents claimed that all treatments were salty (Table 3.9), and

this perception may have affected the overall liking of the products. Fermented kelp had a high rating (~9 on a 12-point scale) for salty taste when subjected to a descriptive sensory test by 13 panelists (Bruhn et al. 2019). In the same way, kelp sauerkrauts were clearly described as saltier than the cabbage control sauerkraut, possibly due to the salty environment in which the kelp are grown. The general saltiness described by the panelists for all the treatments may also have been a result of the 2% NaCl used to produce sauerkraut. The amount of salt added was not adjusted for existing sodium content. Previous research reported that the use of a mineral salt with a low sodium chloride content (57% NaCl, 28% KCl, 12% MgSO₄, 1% SiO₂ and 2% lysine hydrochloride) resulted in a preferred milder tasting sauerkraut as compared to sauerkraut produced with ordinary salt (Viander et al. 2003). Another study reported a positive effect on the sensory quality of sauerkraut with 0.5% salt concentration as compared to 1.5%, 2.5% and 3.5% (Yang et al. 2019). A lower added salt content in the sauerkraut treatments in this study may have increased “overall liking” scores, even beyond 7.1, 6.5, and 6.7 for cabbage, blanched fresh-, and blanched/frozen kelp sauerkraut, respectively, by the more frequent consumers. The addition of kelp in kelp/cabbage sauerkraut significantly reduced the aroma and flavor liking scores as compared to cabbage sauerkraut. The lower responses of participants choosing descriptors such as “salty,” “pungent,” “sour,” “tangy,” “fishy,” “brackish,” and “ocean breeze” for blanched/frozen kelp sauerkraut as compared to blanched fresh sauerkraut could suggest that freezing of samples masked some of these notes of kelp. This is a good indication that freezing could be an alternative preservation method to drying seaweed to enhance product quality for consumers who prefer milder tasting kelp products. The mean acceptability score for kelp sauerkraut

treatments (containing 50% cabbage) for “overall liking” was slightly below 7, which is equivalent to “like moderately,” and indicates promise for acceptance of kelp sauerkraut. The higher overall liking score of the cabbage sauerkraut control was likely due to flavor and aroma, and it suggests that future kelp sauerkraut optimization may be required to increase the sensory score for kelp sauerkraut (> 7.0).

Value addition of seaweed, especially the development of food products appealing to U.S. consumers, will increase their familiarity with seaweed as a food. Such products should be created to increase revenue and satisfy consumers’ changing demands, which are driven by parameters such as population growth, lifestyle and economic changes, and increased awareness about healthy foods.

3.4 Conclusions

With the increase in production of seaweed in the West, data gathered from this research show that kelp could be utilized and consumed as vegetables by consumers. The study revealed that preservation processes had some positive impact on sugar kelp quality and consumer acceptability. Blanching increased greenness but decreased firmness of the kelp. Results from sensory acceptability tests indicate that consumers may like blanched kelp food products more than raw, possibly due to the color change and reduced firmness. Therefore, we can recommend minimal processes such as blanching and freezing for extension of the short shelf life of fresh kelp. Use of such processes will extend marketable life of kelp and may allow preservation for use in formulated foods independent of harvest season. However, costs of water and energy should be considered. Moreover, the absence of pathogens after fermentation in the kelp sauerkraut study

confirms that fermented foods are typically safe, however, proper hygiene and sanitation practices should not be compromised to prevent possible cross-contamination from the environment during and after kelp sauerkraut production. Moreover, freezing can increase kelp retail availability throughout the year and also mask some aroma notes of kelp, such as pungency and fishiness, when it is used to develop value added products. Future studies are warranted to evaluate the impact of extended frozen storage on value added kelp products, since this study focused on the immediate effect of freezing on kelp sauerkraut. Also, blanching, freezing and fermenting kelp into sauerkraut can increase the commercial availability of seaweed products and promote the development of diverse seaweed products that could be easily made at home or conveniently sold in the marketplace year-round. These findings have important implications for the growing U.S. seaweed industry for many economical and nutritional reasons.

CHAPTER 4

EFFECTS OF REHYDRATION TEMPERATURES ON PHYSICOCHEMICAL PROPERTIES AND MICROBIAL QUALITY OF SUGAR KELP (*SACCHARINA LATISSIMA*)

4.1 Introduction

Seaweed is becoming increasingly popular in the West because of its nutritional and functional benefits (Holdt and Kraan 2011; Cornish et al. 2015, 2017), and unique textures and flavors (Figueroa et al. 2021), which make it an important raw material for foods and additives. Seaweed is a seasonal product that contains a large amount of water, with high moisture contents of up to 90% depending on the species (Fudholi et al. 2011; Rode and Dhumal 2017; Sappati et al. 2019). Seaweed is perishable in its fresh state since a high moisture content can facilitate microbial growth. Fresh raw seaweed such as sugar kelp (*Saccharina latissima*) is not suitable for consumption after a week or two of refrigerated storage (Nayyar 2016). Thus, long term preservation methods are necessary to extend its shelf-life. A common long term preservation method utilized in the seaweed industry is drying.

Drying is the removal of moisture or more precisely, the reduction of water activity, resulting in retardation of food spoilage due to an attained physicochemical and microbiological stability (Gupta and Abu-Ghannam 2011; Sablani et al. 2011). Drying, whether open sun drying, predominantly used for seaweed, or other methods such as hot-air oven drying, is an essential step before seaweeds are transported, stored, or used in industrial processing (Gupta and Abu-Ghannam 2011). Recently, there have been a wide

variety of dehydrated seaweed products marketed in the West as snacks, supplements, sushi and ready-to-eat meals. Most of these dehydrated products designed for direct consumption are usually rehydrated by immersion in water or other liquids like sucrose solution (Mastrocola et al. 1998).

Rehydration is a complex process that is intended to restore the properties of the raw (fresh) product by immersing dehydrated products in a liquid phase. During rehydration several changes take place in the material; these are caused by water transfer from the liquid phase into the food and by transfer of soluble solids from the food into the liquid (Lee et al. 2006). Understanding of these mass-transfer mechanisms is important for reliable simulations of rehydration as well as for efficient applications of rehydration at a commercial level. It is important to understand the mass transfer of specific minerals from seaweeds, especially iodine which is very high in *Saccharina latissima*, during rehydration. Lüning and Mortensen (2015) reported a range of 420 – 4000 mg iodine kg⁻¹ dry weight in *Saccharina latissima* from some parts of Europe and Korea, and these iodine levels may negatively affect seaweed consumption since the recommended daily intake and tolerable upper intake levels of iodine in Europe are 0.15 mg/day and 0.60 mg/day, respectively (WHO 2001). However, rehydration of *Saccharina latissima* at room temperature for 5 mins significantly decreased iodine content as compared to the dehydrated products (Correia et al. 2021).

The rehydration characteristics of a dehydrated product can be used as a quality index to reflect the physical, chemical and microbial changes that occurred during drying, and any pretreatment to which the products were subjected (Maskan 2001). Some of these changes may include differences in the product's color, volume, surface area, or

thickness, partial damage to tissue structure, and the destruction of microorganisms or formation of microbial spores as a result of the high temperatures during drying. Changes in the product may influence the ability of rehydration to achieve a high product quality, and may potentially induce a food safety risk from the regrowth of pathogen spores during rehydration. Drying temperatures (35, 50, 60 and 75 °C) differently affected the subsequent rehydration of *Ascophylum nodosum* and *Undaria pinnatifida* (brown seaweeds), with high drying temperatures decreasing the rehydration rate and resulting in a significantly lower moisture content as compared to raw samples (Chenlo et al. 2018). When *Himanthalia elongata* (brown seaweed) was rehydrated at various temperatures (20 – 100 °C) for 80 mins after drying at 40 °C for 24 h, the texture of the seaweed softened significantly during the rehydration process with the greatest reduction in hardness (N/mm) seen at the highest temperature of 100 °C (Cox et al. 2012). Therefore, rehydration temperatures as well as drying temperatures are important to consider in the production of high quality rehydrated products.

To attain a high quality rehydrated seaweed product, most consumers may deem the physicochemical properties such as color and texture of dried food products should closely resemble those of raw (fresh) product to insure consumer acceptability. However, we observed a higher hedonic scores for color during consumer acceptability test for blanched kelp salad treatments due to its greenness (color change) as compared to the golden brown raw kelp salad treatments (Chapter 3). Therefore, data on the impact of rehydration on color and other physicochemical properties is vital to guide seaweed processors to predict consumer acceptability. An informal survey of commercially available dehydrated seaweed products indicated a wide range of suggested rehydration

times (4 – 20 mins) on product labels. Moreover, consumers rehydrate seaweed at various temperatures, or add dried seaweed to soups of various temperatures before consuming. The temperatures of these rehydration liquids, including soups, have a significant impact on product quality (Cox et al. 2012) and consumer acceptability (Pérez-Palacios et al. 2017). Therefore, in this study, rehydration characteristics of sugar kelp (*Saccharina latissima*) were evaluated over a wide temperature range (22, 75 and 100 °C) of industrial interest and also to simulate rehydration practices that may take place at home by consumers. Lang et al. (2016) reported that the survival of three food bacterial pathogens (*Salmonella typhimurium*, *Salmonella enterica* and *Cronobacter sakazakii*) was strongly related to rehydration kinetics of rehydrated milk powder and suggested that a fast rehydration could reduce the drying/rehydration effect on pathogen survival. To assess whether rehydrated kelp products are safe for consumption, the growth patterns of some pathogens, especially spore forming bacteria that can regrow when favorable conditions are attained after rehydration, were evaluated to predict specific situations of potential public-health significance from consuming seaweed. The hypothesis of this study was that rehydration may increase kelp quality and food safety risk. Specifically, this study aimed to determine the physicochemical properties and microbial quality of dried *S. latissima* after subjected to three different initial rehydration temperatures (22, 75 and 100 °C). Results of this study will provide valuable information about the impacts of rehydration temperatures and offer theoretical support for developing appropriate rehydration conditions for the consistent production of high-quality dried *S. latissima* that is safe for consumption.

4.2 Materials and methods

4.2.1 Experimental material and design

Fresh sugar kelp (*Saccharina latissima*) cultivated in South Bristol, Maine, was harvested in June 2021 and donated by Maine Sea Farms (South Bristol, ME, USA). In this study *Saccharina latissima* was dried and then rehydrated using three specific initial rehydration temperatures (Figure 4.1). The focus was on initial water temperatures to more closely reflect kelp rehydration practices at home, where heated water would be added to dried seaweed and left to rehydrate without maintaining a specific temperature. The impacts of the rehydration temperatures on the physicochemical and microbial properties of *S. latissima* were evaluated. Physicochemical and microbial analyses of rehydrated samples were conducted in triplicate unless otherwise stated.

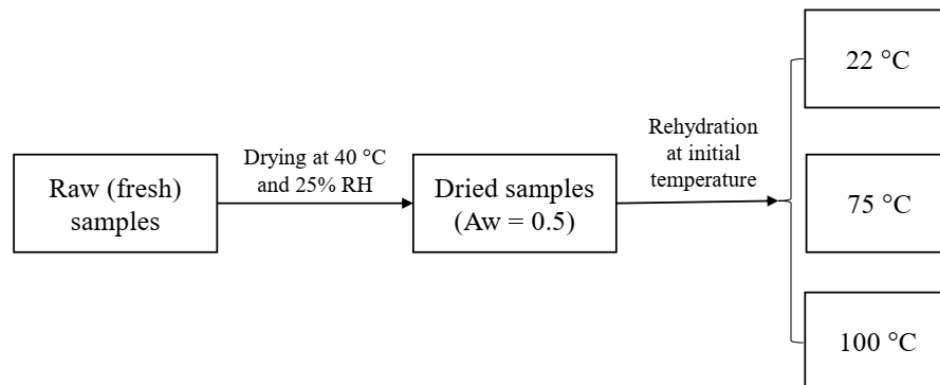


Figure 4.1: Experimental design

4.2.2 Sample preparation

Harvested *Saccharina latissima* (~25 kg) was washed with seawater and received on ice in coolers (Figure 4.2). When samples were received, holdfasts at the end of each kelp blade were cut off and the blades were washed with running tap water to remove the attached biofouling and salts.



Figure 4.2: *Saccharina latissima* in coolers

A few samples of washed *Saccharina latissima* were randomly taken for physicochemical and microbial analysis. The remaining samples were cut horizontally across the kelp blade to provide a length ranging from 20 – 25 cm per sample (Figure 4.3). These cut blades were divided into three groups, each representing a process replicate, and each replicate was dried by hanging the blades on stainless steel grill grates at an air-temperature of 40 °C with relative humidity of 25% and air velocity of 10 m/s using a convective dryer (Cincinnati sub-zero, CSG, OH, USA). Samples were dried to a specific water activity (A_w) ranging from 0.500 to 0.590, similar to water activity values of commercial dehydrated seaweed products previously evaluated in our laboratory (unpublished study).



Figure 4.3: Length of raw (fresh) *Saccharina latissima* for rehydration study

4.2.3 Rehydration

A 1:100 w/v ratio of dry material to water was used to rehydrate the dried *Saccharina latissima* blades. One dried kelp blade at a time was weighed and rehydrated in a rectangular aluminum pan (Handi-foil Corp., Wheeling, IL, USA) of 32.2 cm × 32.2 cm × 10.2 cm dimensions. The appropriate water volume with a starting water temperature of 22, 75 or 100 °C was added to the container to immerse the kelp blade in water for rehydration (Figure 4.4).

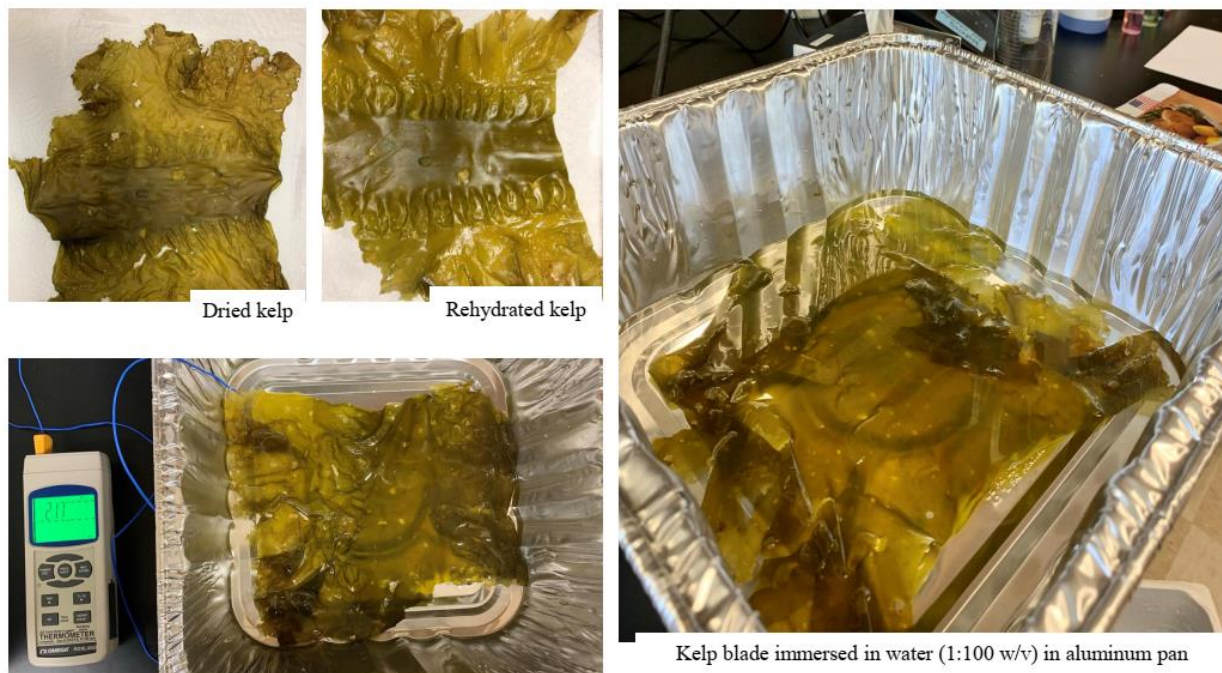


Figure 4.4: Rehydration of *Saccharina latissima*

Each blade was removed from the water every 30 seconds, carefully blotted with paper towels (Bounty, USA) to remove superficial water, weighed (± 0.10 g) and then allowed to continue rehydrating until an equilibrium weight was achieved. Samples were analyzed for physicochemical and microbial properties before and after rehydration. The rehydration ratio of dried *S. latissima* blades was calculated using the equation below (Lewicki 1998):

$$\text{Rehydration ratio (RR)} = \frac{\text{Weight after rehydration (g)}}{\text{Weight before rehydration (g)}}$$

4.2.4 Physicochemical analyses

4.2.4.1 Colorimetric analyses

The color of *Saccharina latissima* before and after rehydration was measured with a colorimeter (LabScan XE, Hunter Labs, USA) fitted with a 5.1 cm diameter aperture, a port size of 5.05 cm, area view of 4.45 cm, and D65 illumination. The colorimeter was

standardized with white and black tiles before each use and was allowed to warm up for 30 min prior to color analysis. Sample blades were placed to cover the bottom of a transparent plastic cup about 60 mm in diameter and 7 mm in height, and L^* , a^* , b^* values were recorded. Two measurements were taken per blade, and three blade samples were analyzed and averaged for each of the three rehydration treatment replicates.

4.2.4.2. *Texture analysis*

For texture analysis, the texture profile analysis (TPA) method was utilized. Three to five circular pieces of raw seaweed and rehydrated *Saccharina latissima* blades (composite of three blades per replicate) of ~6 cm diameter were randomly cut and placed (two layers deep) in the same round transparent plastic cup used for color analysis on the texture analyzer (TA-XTi2, Texture Technologies Inc., Scarsdale, NY, USA) platform. The texture analyzer was calibrated using a 5,000 g load cell before each use. A flat-bottomed plastic cylindrical probe (5 cm diameter) was used to compress the samples twice to 75% strain at a pre-test and post-test speed of 2 mm/s, with a 5 s gap between compressions. Force (Newtons, N), area (N*s), and time (s) were recorded by the texture analysis software (Exponent 32, version 5.0, 6.0, 2010, Texture Technologies Inc.) to calculate the TPA parameters, hardness (maximum force of the first compression), chewiness, and resilience. Hardness was expressed in Newtons (N) and the other TPA parameters are unitless.

4.2.4.3 *pH*

Samples of raw (fresh), dried or rehydrated *Saccharina latissima* (composite of three blades per replicate) were ground and 2 g was placed in a 20 mL cylindrical flask to which 12 mL of de-ionized water was added. Contents were mixed using an agitator

(Thermo Scientific Compact Digital Mini Rotator/Shaker, Pittsburgh, PA) for 1 min. The pH was then measured with a digital pH meter (Benchtop pH / MV Meter – 860031, Scottsdale, AZ) calibrated with standard pH buffer solutions of 4, 7 and 10.

4.2.4.4 Moisture content

Moisture content was determined according to AOAC Method 950.46, by measuring the mass of 5 ± 0.002 g homogenized kelp sample in a pre-weighed aluminum pan and drying at 105 °C for 6 hours (AOAC, 2005) in a convection oven (VWR International, Radnor, PA). All analyses were conducted in duplicate and moisture content expressed in percentage on a wet weight basis (w.b) using the formula below:

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

4.2.4.5 Water activity

The water activity was determined using a water activity meter (AquaLab Decagon, USA) by placing the dried or rehydrated *Saccharina latissima* blades (~1.5 cm diameter) in disposable Aw cups. The water activity meter was calibrated with standard salt solutions with known water activity of 0.750 and 0.900 prior to taking sample reading. All analyses were conducted in triplicate per sample.

4.2.4.6 Water holding capacity (WHC)

Water holding capacity (WHC) is the ability of a food sample to retain its own water even when external pressures, such as heating are applied to it (Huff-Lonergan and Lonergan 2005). WHC analyses for rehydrated kelp was determined according to Jiang et al. (1985). Briefly, 2 g of intact whole blade samples were wrapped in two pieces of pre-weighed Whatman #1 filter paper, placed in 50 mL test tubes, and then spun at 1,000×g for 15 min in a bench top centrifuge (model 5430, Eppendorf, Hamburg, Germany). After

centrifugation, the filter papers were reweighed, and the difference in weight recorded. WHC was calculated as the percent of water retained by the rehydrated seaweed, with respect to water present in the rehydrated sample prior to centrifugation using the following equation:

$$\text{WHC} = \frac{[\% \text{ moisture} \times \text{sample wt. (g)}] - [\text{change in paper wt. (g)}]}{[\% \text{ moisture} \times \text{sample wt. (g)}]} \times 100 \%$$

4.2.4.7 Iodine analysis

Raw (fresh) and rehydrated samples (composite for each rehydration temperature) were freeze dried (VirTis Ultra, USA) at -40 °C, and then ground (Mixer Mill 400, Retsch, Germany) to a particle size of < 300 µm for iodine analysis. Dried samples were also ground prior to iodine extraction according to the method used by Nielsen et al. (2020). Briefly, 0.5 g of ground raw (fresh), dried or rehydrated *Saccharina latissima* were weighed into tubes (Kimax®). Five milliliters Milli-Q® water and 1 mL 25% tetra-methyl-ammonium-hydroxide were added. The tubes were then sealed and placed in a preheated oven at 90 ± 3.0 °C for 3 h followed by cooling and diluting to a final volume of 20 mL with Milli-Q® water. Samples were filtered and analyzed using iCAPTM Q ICP-MS (Thermo Fischer Scientific, Bremen, Germany). The parameter settings were 15.5 L/min coolant gas, 1.1 L/min auxiliary gas, and 0.75 L/min nebulizer gas. Isotopes monitored were 127I and 185Re for internal standard. The limit of quantification (LOQ) for iodine was 37 ug/g and the recovery was 85.4 % (n=3). Here, iodine contents are presented mg iodine/kg (dw) to aid comparison with other studies.

4.2.5 Microbiological analysis

*4.2.5.1 Detection of *Bacillus cereus* and coliforms*

Microbial safety analysis was performed on raw (fresh), dried and rehydrated seaweed samples. A composite sample of three dried or rehydrated blades was analyzed per replicate. To determine pathogens in the kelp, 10 g of each sample were aseptically placed in a stomacher bag containing 90 mL of 0.1% peptone (BD Diagnostics, USA) and stomached for two minutes using a BAGMixer 400 (Model P, Spiral Biotech, Advanced Instruments, Norwood, MA, USA). Using serial dilutions in 0.1% peptone, each sample was plated (1 mL) in duplicate onto 3M petrifilm (3M, Maplewood, MN) for coliform population (35 °C, 24 – 48 h). Additionally, 15 – 25 g of each sample were aseptically placed in a stomacher bag containing nine times (9x) volume (mL) of the initial seaweed weight of mannitol-egg yolk-polymyxin B (MYP) broth (BD Diagnostics, USA) for enrichment (30 °C, 24). Each sample was then plated (0.1 mL) in duplicate onto MYP agar for *B. cereus* ATCC 14579 (30 °C, 24 – 48 h) enumeration, which was obtained from the American Type Culture Collection, Manassas, VA.

4.2.5.2 Enumeration of aerobic plate count (APC) and fungi

Enumeration for aerobic plate count (APC) and fungi (yeast and molds) was the same as described above for coliform with each sample plated (0.1 mL) in duplicate on tryptic soy agar (TSA) (Alpha Biosciences, Baltimore, MD) and acidified potato dextrose agar (APDA) (Alpha Biosciences, Baltimore, MD) with plates inverted and incubated at 37°C for 48h or 25°C for 5 days in the dark, respectively. Dilutions within a countable range (20-200 colonies/15-150 colonies, respectively) were counted using a standard

counting rule. Counts were averaged and recorded as colony forming unit per gram (CFU/g).

4.2.6 Statistical analysis

Data from physicochemical and microbial were analyzed using SPSS 20 (IBM, Armonk, NY, USA) at a significance level of $P \leq 0.05$. One-way analysis of variance (ANOVA) was used to assess all treatment effects and was followed by Tukey's honest significant difference (HSD) post hoc mean separation test at $P \leq 0.05$. A pairwise t-test was used to compare *Saccharina latissima* qualities in each rehydration temperature treatment before and after rehydration.

4.3 Results and discussion

4.3.1 Rehydration ratio

Seaweeds are mostly dried to extend their shelf-life, and dried seaweeds are commonly rehydrated in either hot or warm water, or added to soups before consumption. With many dried seaweed on the market having a water activity (A_w) range of 0.500 to 0.599 from our informal survey, the present study shows the experimental rehydration kinetics of *Saccharina latissima* air-dried with a convective dryer at 40 °C and 25% relative humidity (RH) to attain a A_w range of 0.500 – 0.599. According to Sappati et al. (2019), lower drying temperatures (≤ 50 °C) and lower humidity (25%) are recommended for preserving chemical constituents including total phenolic compounds, and producing a high water holding capacity, respectively, in *S. latissima*. Moreover, drying seaweed at lower temperatures (35 and 50 °C) favored a higher water transfer ratio during rehydration as compared to drying at higher temperatures (60 and 75 °C) (Chenlo et al.

2018). Thus, a low drying temperature (40 °C) and 25% RH were used to produce high quality dried *S. latissima* blades in this study.

Rehydration kinetics showed an initial steep increase in sample mass due to the absorption of water followed by a general decrease in rehydration rate as rehydration progressed (Figure 4.5). The plateau in rehydration ratio was related to the decrease in driving force for water transfer as rehydration progressed. Results indicated that rehydration temperatures did not significantly affect the rehydration ratio in *Saccharina latissima*. These findings contrast with other observed experimental rehydration behavior of food samples including fruits and vegetables subjected to different rehydration temperatures (Krokida and Marinos-Kouris 2003; Krokida and Philippopoulos 2005; Resio et al. 2006), where higher temperatures typically yielded higher rehydration ratios. A similar trend was reported in the rehydration of brown seaweeds, *Ascophyllum nodosum* and *Undaria pinnatifida*, and *Himanthalia elongata*, where higher temperatures increased the amount of absorbed water (high rehydration ratio) (Cox et al. 2012; Chenlo et al. 2018).

High rehydration temperatures may result in swelling of water-holding components (e.g. polysaccharides) in foods, leading to higher water absorption (Tsai et al. 1998). Although the highest initial rehydration temperature (100 °C) did not significantly increase the rehydration ratio (absorbed water) as compared to the initial lower temperatures at the end of the rehydration process (Figure 4.5), it resulted in a significantly higher rehydration ratio (3.10) after 1 min of rehydration as compared to the 22 °C treatment (2.57).

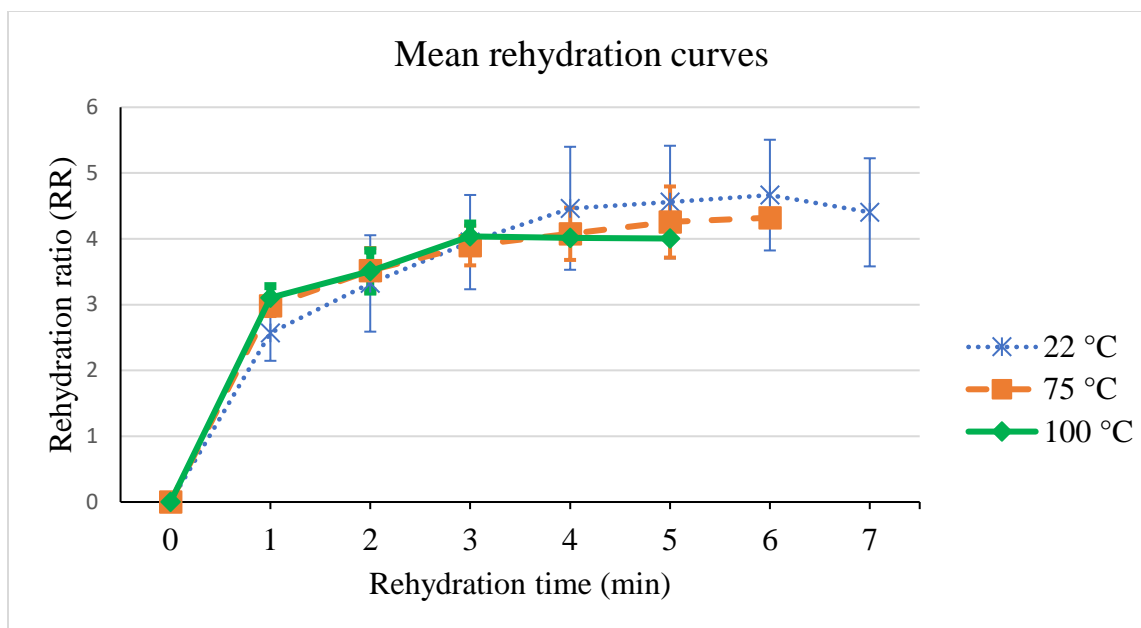


Figure 4.5: *Saccharina latissima* rehydration at 22, 75 or 100 °C (n = 9)

At 1 min, the actual mean temperature for the initial 100 °C treatments was 81.2 °C, which may have been high enough to increase water absorption via swelling of polysaccharides reported to be abundant in *Saccharina latissima* (Wells et al. 2017). However, after 2 minutes the temperature of the water had already decreased to 65.4 °C, which may have limited the extent of swelling. Thus, the lack of significant differences in rehydration ratios among treatments was likely related to the rapid drop in water temperature during rehydration (Table 4.1). The present study applied starting water temperatures of 100 °C and 75 °C which decreased rapidly due to high surface area and the high conductivity of the aluminum pan. The mean starting water temperatures were 22 °C, 74.9 °C and 99.1 °C, and the water temperatures after reaching equilibrium weight were 15.0 °C, 34.1 °C and 44.6 °C, respectively (Table 4.1).

Table 4.1: Average water temperature during rehydration (n = 9)

Time (minutes)		Initial rehydration temperature (°C)		
		22.0	75.0	100.0
Starting temperature	0	22.0	74.9	99.1
	1	21.3	62.7	81.2
	2	20.2	55.5	65.4
	3	19.1	50.2	58.7
	4	18.5	44.8	50.3
	5	17.3	39.3	44.6
	6	16.4	34.1	-
	7	15.0	-	-

The lack of significant differences in rehydration ratio among treatments after the first 2 min of rehydration was similar to results reported during the rehydration of the brown seaweed, *Himenthalia elongata* (Cox et al. 2012). In that study, rehydration ratios of *H. elongata* subjected to 100 °C, 80 °C and 60 °C water temperatures were not significantly different from the 20 °C treatment as determined after 55-80 min of rehydration.

The highest initial water temperature (100 °C) resulted in a shorter rehydration time (5 min) to achieve equilibrium weight as compared to the 75 °C and 22 °C treatments at, 6 and 7 min, respectively. Similarly, a higher rehydration temperature (70 °C) for dried *Boletus edulis* mushrooms produced a shorter rehydration time (66.67 min) as compared to 20 °C (116.67 min) (Hernando et al. 2008). In brown seaweed, *Himenthalia elongata*, the highest rehydration temperature (100 °C) resulted in a shorter time (30 min) for equilibrium as compared to the lowest rehydration temperature (20 °C) which required 70 min (Cox et al. 2012). This trend suggests that high temperatures facilitate water absorption and mass transfer in and out of food samples, including seaweed, faster than lower temperatures, which follows the findings of mass transfer

phenomena where volumetric liquid mass transfer coefficient increases as temperature is increased (Ferreira et al. 2010).

4.3.2 Color

The surface color of food is a quality attribute that is commonly affected by processing. Thermal processing, in particular, can severely alter surface color due to chemical and enzymatic degradation of pigments (Perera 2005). In turn, some of these degradations may be minimized during rehydration based on the water temperature. Drying and rehydration significantly ($P \leq 0.05$) increased L^* values, while rehydration decreased a^* values of *Saccharina latissima* as compared to raw samples (Table 4.2). A previous study on drying of *S. latissima* by convective-air oven at 30 °C and 50 °C with 25% relative humidity (RH) reported a significant increase in L^* and b^* values and a decrease in a^* values (Sappati et al. 2019). However, only L^* values significantly increased after drying at 40 °C and 25% RH in the present study (Table 4.2), which denotes the influence of heat on surface lightness. It is important to note that all kelp samples were dried with the same drying parameters to an expected water activity (0.5) but they were processed in three separate groups for subsequent rehydration treatments.

Table 4.2: Color (Hunter L*, a*, b*) of raw (fresh), dried, and rehydrated *S. latissima* (mean \pm SD)

Treatment	L*	a*	b*
Raw (fresh)	18.89 \pm 0.98 ^B	4.42 \pm 0.83 ^A	11.44 \pm 1.04 ^{AB}
Dried for 22 °C rehydration	25.54 \pm 3.18 ^A	3.94 \pm 2.67 ^{AB}	11.31 \pm 4.66 ^{AB}
Dried for 75 °C rehydration	24.54 \pm 2.51 ^A	2.50 \pm 1.71 ^{ABC}	10.29 \pm 2.33 ^B
Dried for 100 °C rehydration	25.14 \pm 1.61 ^A	4.24 \pm 2.27 ^A	9.72 \pm 3.15 ^B
Rehydrated at 22 °C	24.70 \pm 3.07 ^A	1.13 \pm 1.24 ^{BC*}	16.27 \pm 2.96 ^{A*}
Rehydrated at 75 °C	26.58 \pm 2.03 ^{A*}	0.46 \pm 1.01 ^{C*}	14.34 \pm 3.80 ^{AB*}
Rehydrated at 100 °C	26.94 \pm 4.12 ^A	0.63 \pm 1.13 ^{C*}	13.45 \pm 2.62 ^{AB*}

Raw (fresh) samples (n = 3), dried, and rehydrated samples (n = 9)

Capital letters denote significant differences among treatments; asterisk denotes significant difference before and after rehydration (pairwise t-test).

Hunter (L*, a*, b*): L* = lightness, a*= red/green, b*=yellow/blue.

There were no significant differences in color (L*, a*, b* values) among the samples rehydrated at different temperatures. This may be as a result of the high standard deviations observed and the rapid decrease in initial water temperature during the rehydration process. Notably, a pairwise t-test comparison of dried and rehydrated samples showed a significant decrease and increase ($P \leq 0.05$) in a* and b* values, respectively, after rehydration irrespective of the rehydration temperature. Results suggest that some water-soluble pigments that give sugar kelp its brown color may have leached out during rehydration, although the predominant pigment, fucoxanthin, in sugar kelp is lipid-soluble. The decrease in a* values in higher temperature rehydrated samples, indicative of increased greenness of *Saccharina latissima*, validates the fact that heat breaks down the brown pigment, fucoxanthin, in brown seaweed as reported in previous chapters (Akomea-Frempong et al. 2021a, b). The increase in the greenness of kelp after rehydration may increase consumer acceptability (Akomea-Frempong et al. 2021b), since

color is one of the key factors behind consumers' decisions to buy a particular food (Barrett et al. 2010). There were significant changes in L* and a* values of rehydrated samples, thus, none resembled the raw kelp samples in terms of color although L* and a* values of rehydrated kelp subjected to an initial water temperature of 22 °C were slightly closer to those of raw samples than the 75 °C and 100 °C treatments (Table 4.2). In contrast, betel (*Piper betel* L.) leaves rehydrated at temperatures of 25 °C and 40 °C mostly resembled fresh leaves in color compared with leaves that were rehydrated at 80 °C (Balasubramanian et al., 2011).

4.3.3 Texture

Mass transfer during rehydration is a very important process that affects the quality and utilization of many food products, especially their cell structure and food matrix. An important attribute of food products is their porosity, which is affected by drying and rehydration temperatures (Mayor and Sereno 2004). A higher drying temperature may increase the shrinkage stress of dried plant tissues and lead to larger pores, and if the pores are found in the inner portion of the product with entrapped air, it will prevent the absorption of rehydrating water (Witrowa-Rajchert and Lewicki 2006). Therefore, a low drying temperature of 40 °C was employed in this study to overcome such challenges. In the present study, it was observed that the fresh sample showed higher hardness (N) and chewiness values but its tissue presented less resilience (Table 4.3).

Table 4.3: Instrumental texture analysis of raw and rehydrated *Saccharina latissima* [mean \pm SD (n = 3)]

Treatment	Hardness (N)	Chewiness	Resilience
Raw (fresh)	77.47 \pm 14.63 ^A	20.71 \pm 2.59 ^A	0.41 \pm 0.09 ^C
Rehydrated at 22 °C	54.43 \pm 11.01 ^{AB}	11.79 \pm 2.50 ^B	0.55 \pm 0.07 ^{BC}
Rehydrated at 75 °C	48.30 \pm 6.33 ^B	11.97 \pm 1.39 ^B	0.71 \pm 0.09 ^{AB}
Rehydrated at 100 °C	43.45 \pm 9.74 ^B	12.47 \pm 1.66 ^B	0.82 \pm 0.02 ^A

Raw (fresh) samples (n = 3), dried and rehydrated samples (n = 3)

Capital letters denote significant differences among treatments.

N = Newtons

Chewiness decreased in all rehydrated samples, while hardness decreased in 75 °C and 100 °C treatments, as compared to raw kelp samples. Hardness values after rehydration were similar to other food rehydration studies including vegetables such as pepper (Heredia-Léon et al. 2003) and brown seaweed, *Himenthalia elongata* (Cox et al. 2012). Although rehydration with the highest initial water temperature (100 °C) did not significantly decrease hardness as compared to the lowest initial water temperature (22 °C), the relatively lower hardness of the 100 °C samples (Table 4.3) suggests more damage of kelp tissues that may have promoted a significant loss of mechanical resistance during rehydration. Also, during rehydration, sugars solubilize and molecules become more mobile, which can increase solids loss throughout processing. These effects correlate positively with increased temperature (Witrowa-Rajchert and Lewicki 2006). The lower hardness and chewiness values of the rehydrated samples as compared to raw kelp may promote increased consumer acceptability, since sensory participants in our previous kelp salad study preferred less firm and chewy kelp samples (Akomea-Frempong et al. 2021b). Moreover, the significantly higher resilience value in the 100 °C rehydrated samples (0.82 \pm 0.02) as compared to the 22 °C and 75 °C samples (0.55 \pm 0.07 and 0.71 \pm 0.09, respectively) suggest that the higher temperature may have

solubilized more components in the kelp, thereby concentrating more of the insoluble components to allow rehydrated kelp to recover from deformation more readily after compression by the probe.

4.3.4 Moisture, water activity, water holding capacity, and pH

Most rehydrated food products have decreased hydrophilic properties and lower water absorption capacity due to rupture and dislocation of cellular structure and shrinkage of capillaries (Krokida and Philippopoulos 2005). Thus, dried product generally does not regain its original properties after rehydration. In the present study, rehydrated samples had significantly ($P \leq 0.05$) lower moisture content (73.2-85.9%) after achieving equilibrium weight as compared to raw samples (89.8%) (Table 4.4). All the moisture contents of rehydrated samples in this study were lower as compared to those of *Saccharina latissima* rehydrated in water at room temperature for 5 minutes ($93.0\% \pm 0.1$) (Correia et al. 2021).

Table 4.4: Moisture, water activity and water holding capacity (WHC) of raw (fresh), dried and rehydrated *S. latissima* (mean \pm SD)

Treatment	Moisture (%)	Water activity	WHC (%)
Raw (fresh)	89.8 ± 0.9^A	0.929 ± 0.030^A	90.8 ± 1.8^A
Dried for 22 °C rehydration	-	0.530 ± 0.028^C	-
Dried for 75 °C rehydration	-	0.546 ± 0.029^C	-
Dried for 100 °C rehydration	-	0.538 ± 0.023^C	-
Rehydrated at 22 °C	85.9 ± 0.2^B	$0.892 \pm 0.015^{AB*}$	80.7 ± 4.3^B
Rehydrated at 75 °C	84.5 ± 0.9^B	$0.859 \pm 0.031^{B*}$	83.1 ± 7.0^{AB}
Rehydrated at 100 °C	73.2 ± 11.7^C	$0.858 \pm 0.031^{B*}$	85.6 ± 8.1^{AB}

Raw (fresh) samples (n = 3), dried and rehydrated samples (n = 9)

Capital letters denote significant differences among treatments; asterisk denotes significant difference before and after rehydration (pairwise t-test).

The same trend was reported in rehydration studies of other fruits and vegetables including seaweed (*Himenthalia elongata*), where rehydrated samples had moisture

contents below those of raw samples (Maskan 2001; Cox et al. 2012; Chenlo et al. 2018). The significantly lower moisture content of samples from the 100 °C treatment (73.2%) as compared to 75 °C and 22 °C (84.5% and 85.9%, respectively) may be as a result of the high standard deviation recorded for the 100 °C samples. The high standard deviation of those samples may have been due to the high variability in kelp texture and relatively shorter average rehydration time for *Saccharina latissima* to reach equilibrium weight (Figure 4.5).

Rehydration at different temperatures did not significantly affect the water activity (A_w) of the kelp blades (Table 4.4). The A_w of rehydrated samples was significantly lower than that of raw samples (0.929), except for the 22 °C rehydrated samples (0.892). This indicates that a higher rehydration temperature may be relatively better for promoting kelp microbial quality and safety. However, the A_w data denote that there was sufficient free water in *Saccharina latissima* after rehydration to favor microbial growth. Thus, consuming or processing *S. latissima* immediately after rehydration is necessary to minimize microbial growth since an A_w of 0.65 or below is required to limit growth of bacteria and fungi (mold and yeast) (Krokida and Philippopoulos 2005; Labuza and Altunakar 2007). A_w is also an important factor with regard to maintaining the stability of pigments in food, and the A_w of rehydrated kelp is sufficiently high to influence the rate of acid-catalyzed degradation of chlorophyll to pheophytin, a brown discoloration that can alter product quality (Von Elbe, 1987).

The lower A_w values of rehydrated samples as compared to raw may be as a result of case-hardening during the drying of kelp blades. According to Heldman (2013), case hardening is very common in dehydrated foods since along with the moisture, soluble

solids migrate to the food surface and form an impervious layer that creates a situation where the inner moisture is trapped by the hard outer surface. This restricts matrix mobility and entraps air, contributing to more closed pore formation within the food material (Achanta et al. 1997; Gulati and Datta 2015), which may lead to lower water absorption during rehydration.

The water holding capacities (WHC) of rehydrated samples were not significantly different from each other (Table 4.4). However, the WHC of 22 °C samples (80.7%) was significantly lower than WHC of raw samples (90.8%). The decrease in WHC in rehydrated samples (80.7-85.6%) as compared to raw samples suggests a structural breakdown in *Saccharina latissima* blades during drying, which decreased their water holding capacity. The higher rehydration temperature resulted in a higher WHC although it was not statistically different from the lower rehydration temperatures. The higher rehydration temperature samples absorbed less water than the other samples, but were better able to hold onto that water under the force of centrifugation.

The pH values (Table 4.5) significantly decreased in dried kelp blades (6.17) as compared to raw samples (6.84), similar to the pH trends observed in dried tomatoes, mango (Das Purkayastha et al. 2013; Kumar and Sagar 2014) and *S. latissima* (raw = 6.67, dried at 30 °C – 50 °C = 6.24 – 6.26) (Sappati et al. 2019). The 100 °C rehydration treatment resulted in a significantly lower pH (6.37) as compared to the 22 °C rehydration treatment (6.71). It is possible that the 100 °C water temperature degraded some components of the kelp blade, releasing organic acids or H⁺ that made the samples from 100 °C rehydration slightly more acidic. Also, pH values of the kelp samples rehydrated at 75 °C and 100 °C were significantly lower than in the fresh sample, likely

due to the relatively higher concentration of solids in the rehydrated kelp samples as rehydration temperature increased.

Table 4.5: pH and iodine contents of raw (fresh), dried, and rehydrated *Saccharina latissima* (mean \pm SD)

Treatment	pH	Iodine mg/kg (dw)
Raw (fresh)	6.84 \pm 0.14 ^A	6,227 \pm 21
Dried samples	6.17 \pm 0.09 ^D	2,652 \pm 21
Rehydrated at 22 °C	6.71 \pm 0.05 ^{AB}	1,206 \pm 21
Rehydrated at 75 °C	6.50 \pm 0.11 ^{BC}	1,053 \pm 21
Rehydrated at 100 °C	6.37 \pm 0.14 ^{CD}	1,306 \pm 21

pH: (n = 3)

Iodine: Raw (fresh) sample (n = 1, from one whole blade), dried and rehydrated (n = 1, composite samples). Each sample was analyzed 3 times. Capital letters denote significant differences among treatments.

4.3.5 Iodine content

The relatively lower utilization of *Saccharina latissima* as compared to other seaweeds like *Porphyra/Pyropia/Neopyropia/Nori* for culinary purposes in Europe may be related to its high iodine levels (3,000 – 10,000 mg/kg dw) (Holdt and Kraan 2011). According to Nielsen et al. (2020) and Lüning and Mortensen (2015), unprocessed *Saccharina latissima* contains as much as 4,605 mg/kg (dw) depending on the site of cultivation, which raises concerns among various food regulatory bodies since the recommended daily intake of iodine in Europe is 0.15 mg/day (WHO 2001).

In the present study, iodine content (Table 4.5) in raw *Saccharina latissima* (6,227 \pm 21 mg/kg (dw)) was somewhat higher than iodine content in raw samples reported in other studies (Roleda et al. 2018; Stévant et al. 2018b; Nielsen et al. 2020). It is important to note that due to high analytical cost, only one raw kelp blade was analyzed for iodine content, as compared to the dried and rehydrated treatments values,

which represent composite samples of 9 blades. All composite samples were analyzed in triplicate. The high level of iodine in the raw sample may have been due to the persistently submerged biomass of *S. latissima* in Maine (U.S.), regardless of the cultivation system used (Roleda et al. 2018). When kelp are submerged in water with limited external stress, they accumulate iodide from seawater, but release iodide to scavenge reactive oxygen species (ROS) when environmental factors such as low tides that expose the kelp incite oxidative stress (Küpper et al. 1998). The iodine concentration in kelp may also depend on the geographical area in which the kelp was cultivated or may be impacted by seasonal effects, but further study is warranted to support these assumptions (Lüning and Mortensen 2015).

Rehydrated *Saccharina latissima* samples from all treatments presented a reduced concentration in iodine (~39 - 49% of original values) when compared to iodine content in dried kelp and ~79 – 83% as compared to raw kelp. Another *S. latissima* rehydration study reported 78.0% and 93.0% iodine concentration reduction when raw kelp samples were rehydrated at 30 and 60 °C, respectively, for five minutes (Nielsen et al. 2020). In their study, iodine content negatively correlated with increased rehydration temperature and time. In the present study, there were no notable differences in iodine content of samples among rehydration treatments, which ranged from 1053 – 1306 mg/kg (dw). However, although there was no apparent trend between rehydration temperatures and iodine content (Table 4.5), results confirm that rehydration can significantly reduce a substantial amount of the original iodine content. Kelp processors should consider optimizing dried sugar kelp rehydration processes to better predict reduction in iodine content, to better inform consumers and to reduce the risk of consuming high iodine

levels. However, rehydration methods should be considered especially with regard to home consumers, since rehydration parameters are not standardized at home as in rehydration that takes place in the industry. Also, the addition of kelp to soups for flavor prior to consumption will result in consuming the same high amount of iodine from the rehydrated kelp and the iodine-rich broth (Zava and Zava 2011).

4.3.5 Microbiological analysis

Bacterial spores are of concern to the food industry due to their ability to survive various processes designed to kill their vegetative cells, and their potential to subsequently germinate and grow in food (Daelman et al. 2013). Some of these spores including those from *Bacillus* species can cause food spoilage or foodborne disease. Some *Bacillus* species have been isolated from a wide variety of foods including seaweed (Singh et al. 2011) and are generally recognized as ubiquitous in nature and particularly in a marine environment (Liu et al. 2017). In this study, one replicate (33.3%) out of the three raw sample replicates tested positive for *B. cereus*. All dried and rehydrated samples were negative for *B. cereus*, except for one positive result out of the three 100 °C rehydrated samples. Similarly, *B. cereus* was not detected in dried *Saccharina latissima* from west Spain (del Olmo et al. 2018) or dried ready-to-eat *Laminaria* spp. in Italy (Martelli et al. 2021), but was detected in other seaweed species. The presence of *B. cereus* in a rehydrated sample suggests that regrowth of *B. cereus* is possible, although sporadic, and can be a potential risk for consumers.

Apart from raw samples, coliform counts were below the detection limit (1.00 log CFU/g) in all treatments (Table 4.6), which suggests that heat applied during drying may have killed all vegetative cells. Coliform results also imply that the seaweed was

processed (drying and rehydration) under good sanitation practices as coliform bacteria is of fecal matter origin (Schwaiger et al. 2012).

Table 4.6: Enumeration of APC, fungi, and coliform for raw (fresh), dried and rehydrated *Saccharina latissima* [log CFU/g (mean \pm SD)]

Treatment	APC	Fungi	Coliform
Raw (fresh)	3.41 \pm 0.25 ^A	2.63 \pm 0.31 ^A	1.82 \pm 0.31 ^A
Dried samples	2.68 \pm 0.80 ^A	2.00 \pm 0.00 ^B	\leq 1.00 \pm 0.00 ^B
Rehydrated at 22 °C	3.25 \pm 0.11 ^A	2.10 \pm 0.17 ^{AB}	\leq 1.00 \pm 0.00 ^B
Rehydrated at 75 °C	3.08 \pm 0.16 ^A	2.16 \pm 0.28 ^{AB}	\leq 1.00 \pm 0.00 ^B
Rehydrated at 100 °C	3.08 \pm 0.16 ^A	2.10 \pm 0.17 ^{AB}	\leq 1.00 \pm 0.00 ^B

Raw (fresh), dried and rehydrated samples (n = 3)

Detection limit for APC and fungi = 2.00 log CFU/g, and coliform = 1.00 log CFU/g

Capital letters denote significant differences among treatments.

Drying and rehydration did not significantly ($P \geq 0.05$) affect aerobic plate count (APC). A relatively low APC (< 3.5 log CFU/g) was found for all treatment samples (Table 4.6) which were comparable to APC of raw samples (2.2 -3.4 log CFU/g) evaluated in the previous chapters (Akomea-Frempong et al. 2021a, b). Similarly, *Saccharina latissima* harvested at two different times and refrigerated for 10 days had similar APC counts ranging from 3.08 – 5.64 log CFU/g. Drying significantly ($P \leq 0.05$) reduced fungi counts suggesting the impact heat has on fungi. Fungi counts in rehydrated samples (2.10 – 2.16 log CFU/g) were similar to fungi counts detected in raw *S. latissima* and those frozen for a year (2.00 – 2.14 log CFU/g), reported in Chapter 2.

4.4 Conclusions

Rehydration is common in the consumption and processing of seaweed since most are dried to extend their shelf-life. Rehydrating dried seaweeds, kelp in particular, to achieve their initial product quality may not be attainable due to the impact of drying on

seaweed microstructure. However, rehydration procedures that ensure the safety of food products while restoring product qualities close to those of raw samples are important to the seaweed industry and consumers alike. Our findings revealed that rehydration temperatures (22 °C, 75 °C and 100 °C) did not affect the rehydration ratio of *Saccharina latissima*, possibly due to the lack of consistent rehydration water temperature throughout the process. Future rehydration studies should consider taking steps to minimize the rapid decline of water temperature, although that may not be representative of consumer practices in the home. Rehydration treatments did not have a significant impact on A_w , WHC, hardness, chewiness, color parameters and a number of microorganisms evaluated. However, the highest initial water temperature (100 °C) resulted in a shorter time for kelp to reach equilibrium weight, and these samples had higher textural resilience and lower moisture content, which may impact their consumer acceptability when used in prepared dishes. However, particular attention should be given to rehydration at 100 °C, as that condition may favor erratic *Bacillus cereus* spore regermination. Notably, iodine content significantly decreased after rehydration in all treatments, which may be advantageous for growers and kelp producers seeking to promote health benefits of value added sugar kelp products. In perspective, evaluating other valuable compounds such as minerals and antioxidant activity in rehydrated sugar kelp will help produce high quality rehydrated sugar kelp. Also, conducting a sensory evaluation of rehydrated product is an area worth pursuing.

CHAPTER 5

DETECTION AND SURVIVAL OF *LISTERIA MONOCYTOGENES*, *VIBRIO* SPP., *SALMONELLA* SP., AND SHIGATOXIGENIC *ESCHERICHIA COLI* ON SUGAR KELP (*SACCHARINA* *LATISSIMA*) DURING STORAGE

5.1 Introduction

Seaweed has been part of the human diet for many thousands of years (Dillehay et al. 2008) and the sustainability in production and high nutritional content of edible seaweeds including kelp (Holdt and Kraan 2011) has led in part to an increase in their production globally (Grossart et al. 2006; Caponigro et al. 2010; Kim et al. 2017). Seaweed production includes the increase in seaweed aquaculture to supplement the wild harvest, which in the U.S. is predominant in the northeast regions and west coast of the country (Kim et al. 2019b; Piconi et al. 2020). Kelp contributes about 90% of seaweed produced in the U.S. (Piconi et al. 2020) and has high levels of dietary fiber, minerals and antioxidant activities, attributed to its content of polyphenolic compounds (Holdt and Kraan 2011; Stévant et al. 2018a). Kelp is currently being utilized in many food applications and are consumed as sea vegetables by consumers as well (Akomea-Frempong et al. 2021b).

Food safety data shows that vegetables have been implicated in foodborne disease outbreaks caused by a variety of pathogenic microorganisms (Machado-Moreira et al. 2019; Bennett et al. 2021). As a result, several studies have been conducted to determine the incidence of microorganisms such as *Vibrio* spp., *Escherichia coli*, *Listeria*

monocytogenes, and *Salmonella* spp. (Sant'Ana et al. 2012; Tango et al. 2018; Zhang et al. 2020), among others, in different types of vegetables.

The contamination of vegetables including seaweed can occur either at the production (growing/harvest) site or during handling or processing (Caponigro et al. 2010; Barberi et al. 2019). There are numerous bacterial pathogens persisting in coastal and estuarine waters where seaweed grows, thus a greater possibility that edible seaweeds may become contaminated. Recent human activities have increased water temperatures (Turner et al. 2009; Wernberg et al. 2019; Bricknell et al. 2021) and ocean acidification in marine ecosystems leading to a decrease in pH (Woosley et al. 2016; Donham et al. 2021). These conditions resulted in an increase in the production of protease and glycosidase in the water environment (Grossart et al. 2006), which elevates marine bacterioplankton associated with the pathogenicity of some microorganisms (Ridgway et al. 2008). Some of these bacterial pathogens such as *Vibrio* spp. (Newton et al. 2012) that naturally inhabit or are prevalent in estuarine and coastal waters, have been implicated in foodborne illness in the U.S. (Stentiford et al. 2022).

Vibrio is a genus of Gram negative, rod-shaped bacteria with roughly a dozen species known to cause disease in humans (Austin 2010). The infection is usually from exposure to seawater or consumption of raw or undercooked seafood (Newton et al. 2012). In 2014, infection resulted in an estimated 1,252 *Vibrio* infections (excluding toxigenic *V. cholerae* O1 and O139) that were reported to cholera and other vibrio illness surveillance (COVIS), with about 326 were hospitalized, and 34 deaths (CDC 2014). The most common pathogenic species are *V. cholerae*, *V. parahaemolyticus*, and *V. vulnificus*, with non-cholera *Vibrio* spp. causing vibriosis. An increased growth of *Vibrio*

spp. has been associated with high water temperatures (Turner et al. 2009; Baker-Austin et al. 2013), suggesting that the increasing global ocean temperatures may pose an emerging *Vibrio* risk from food products grown in marine ecosystems (Baker-Austin and Oliver 2018; Deeb et al. 2018; Hackbusch et al. 2020). Although cholera cannot be considered likely to be associated with seaweed, an unusual case was reported where a woman got infected with cholera after she consumed raw seaweed contaminated with *V. cholerae* a month after transporting the seaweed via her luggage from the Philippines to her home in California, U.S. (Vugia et al. 1997). This case is rare and should be prevented by laws prohibiting the transport of raw and fresh vegetables and fruits from other countries by tourists.

Agricultural runoff waters and untreated waters may contain bacterial pathogens which can cause foodborne illness too. Among these pathogens are Shigatoxin-producing *Escherichia coli* (STEC), which have been implicated in several foodborne outbreaks (CDC 2020a, b). This contaminated runoff can end up in the oceans and estuaries where seaweeds are grown, and may increase the risk of STEC contamination in products including shellfish and seaweeds. The most common Shigatoxin-producing serotypes in North America include O157, O26, O111, O103, O45, and O121. The CDC estimates approximately 176,000 illnesses, 2,400 hospitalizations, and 20 deaths per year in the United States from pathogenic *E. coli* (Scallan et al. 2011).

Additionally, other bacterial pathogens such as *Listeria monocytogenes* and *Salmonella* spp. occasionally contaminate fresh produce during and after harvesting, which can present a serious health risk in minimally processed vegetables including seaweed. *Listeria monocytogenes* are Gram positive, non-spore forming, facultatively

anaerobic rods that can grow at lower temperatures (psychrotrophic) (Ryser and Buchanan 2013). *Listeria* species are commonly found in agricultural environments, on processing equipment, and raw and unprocessed food products. Major outbreaks of listeriosis, with high morbidity and mortality, have been caused by a variety of foods, including vegetable products (Zhu et al. 2017). Food processing settings may provide a conducive environment for *Listeria* due to the cooler temperatures and presence of moisture (Camargo et al. 2017). Hence, fresh seaweed with high moisture content that requires lower temperatures during storage and processing could be at risk of *L. monocytogenes* contamination.

Moreover, *Salmonella* spp., Gram negative bacteria of animal origin are ubiquitous in soil, water and vegetation (Ferrari et al. 2019). Recently, *Salmonella* infection outbreaks associated with the consumption of raw or minimally processed fruits and vegetables have increased (Quiroz-Santiago et al. 2009; Bennett et al. 2021). The factors influencing the increase in salmonellosis outbreaks due to vegetables include, but are not limited to, changes in agricultural practices, poor handling, and processing conditions of fresh produce (Wadamori et al. 2007), which may include seaweed.

Currently, the seaweed-producing regions in the U.S. do not have unified established regulations for farm sites, seaweed production, and post-harvest practices such as those put in place by the National Shellfish Sanitation Program (NSSP) of the U.S. Food and Drug Administration (FDA) for shellfish production (FDA 2019). Also, macroalgae (seaweed) are not approved by the U.S. FDA as produce (FDA 2018d), therefore fresh seaweed cannot be strictly subjected to the Food Safety Modernization Act (FSMA) final rule on produce safety (FDA 2018d). This could result in an increased

risk of bacterial pathogen contamination of fresh seaweed produced, processed, and consumed in the U.S. Seaweed processors of qualifying scale in the U.S. are required to implement a food safety plan like the preventive controls to safeguard seaweed production and minimize food hazards, but this requirement does not extend to growing and harvesting activities.

Notably, there have been few reported bacterial pathogens detected on seaweed. Diverse *Vibrio parahaemolyticus* and *V. vulnificus* populations were detected on ‘*Porphyra*’ (*Pyropia/Neopyropia*), *Undaria* and ‘*Laminaria*’ (*Saccharina*) species harvested throughout the year in Japan (Mahmud et al. 2007, 2008). Kimbab, a popular ready-to-eat food in Korea made of several ingredients including rice and seaweed (nori), tested positive for *Salmonella* spp. (36.7%) and *Listeria monocytogenes* (6.7%) out of the 30 samples tested (Cho et al. 2008). In Turkey, *Vibrio* spp. (<10 CFU/g) were reported in samples of sundried *Ulva lactuca* (Karacalar and Turan 2008) and in Maine (U.S.), *E. coli* O157:H7, *Vibrio* spp. and *Salmonella enterica* ser. Typhimurium, were detected on *Saccharina latissima* produced at non-approved areas for bivalve aquaculture (Barberi et al. 2019). In 2016, fifteen cases of salmonellosis were linked to seaweed from an aquaculture farm in Oahu, Hawaii, where *Salmonella enterica*, serovar Weltevreden was detected in 1 and 10 samples out of the 12 seaweed and 36 water samples tested, respectively (Nichols et al. 2017). These instances reinforce the possibility of unapproved sites and poor sanitation serving as a source of contamination to seaweed products.

Despite the presence of these bacterial pathogens on seaweed, the ability of these microbes to survive, grow and cause disease depends on their survival during minimal processing and storage (Capozzi et al. 2009), the interactions between the host (seaweed)

and the pathogen, and the natural microflora of the host (seaweed) and the pathogen (Brandl 2006). Seaweeds are considered to be a potential source of secondary metabolites with wide variety of biology activity, including antialgal, antibacterial, antiviral and antifouling activities (Lubobi et al. 2016; Pérez et al. 2016; Sun et al. 2019). Currently there are no published studies on the survival and growth of bacterial pathogens on *Saccharina latissima*. Temperature control is critical to food safety (Söderqvist et al. 2017), but the recommended refrigeration temperature for perishable foods including salads and vegetables varies among different countries. The FDA advises 4 °C in the U.S. (FDA 2021), and maximum refrigerated temperature in Denmark, Finland and Sweden is 5 °C, 6 °C and 8 °C, respectively (Møller et al. 2016), with various temperature abuses observed in domestic refrigerators (EFSAPBH 2012). Thus, the aim of this study was to determine the survival of inoculated *Vibrio* spp., shigatoxigenic *Escherichia coli* (STEC), *Listeria monocytogenes* and *Salmonella* sp. on sugar kelp subjected to different temperatures during post-harvest storage.

5.2 Materials and methods

5.2.1 Experimental design

Sugar kelp (*Saccharina latissima*) was grouped into two product forms, whole blade and shredded slaw. Each product form was inoculated with four bacterial pathogens each and stored at 4 °C and 10 °C for 7 days, and 22 °C for 8 hours. The samples were evaluated immediately one-hour post inoculation for all three temperatures (time 0), then either every day for samples stored at 4 °C and 10 °C, or every 4 hours for samples stored at 22 °C. Each treatment was processed in triplicate.

5.2.2 Sample preparation

Fresh sugar kelp sourced from Maine Sea Farms (South Bristol, Maine, USA) in June 2021 was washed with tap water to remove debris, epiphytes, and fouled tissues. Holdfasts of sugar kelp were removed, and the kelp was grouped into two treatments. The first group were whole blades that were cut horizontally across the blade into sections weighing 25 ± 0.30 g prior to inoculation and stored in resealable zipper plastic bags (Hannaford Gallon Recloseable Freezer Bags) each at appropriate temperature. The other group was shredded with a food processor (RobotCoupe®, CL 50 Series E, Jackson, MS, USA) fitted with a 1/8" slicing disc to produce shreds ranging from ~2-5 mm in width and ~5-25 cm in length. About 25 ± 0.30 g of shredded slaw were inoculated prior to storage them in the resealable zipper plastic bags each at appropriate temperature.

5.2.3 Bacterial inoculum preparation

A single colony of *Escherichia coli* O111:H8 ATCC BAA 184, *E. coli* O26:H11 ATCC BAA-1653, *Vibrio parahaemolyticus* ATCC 17802, *Vibrio vulnificus* ATCC 27562, *L. monocytogenes* ATCC 19111, *L. monocytogenes* ATCC 19115 as well as *Salmonella* Enteritidis ATCC BAA-1045 (all sourced from American Type Culture Collection, Manassas, VA), and *Salmonella* Saintpaul LHH-1311-1 (a walnut isolate identified by the Waite-Cusic lab at Oregon State University), were used in this study. Bacterial inoculum preparation for sugar kelp followed the method used by Callahan and Perry (2020) with slight modifications. Briefly, each strain of *L. monocytogenes*, *E. coli*, and *Salmonella* and each species of *Vibrio*, previously stored frozen at -80 °C was individually cultured in non-selective broth overnight, streaked on tryptic soy agar (TSA,

Alpha Biosciences, Baltimore, MD) and incubated at optimal growth temperatures (Table 5.1) to ensure cultures were not contaminated.

Table 5.1: Selective broth and incubation temperature used

Pathogen	Selective broth	Incubation Temperature (~12 hours)
<i>Listeria monocytogenes</i>	TSB	30 °C
<i>Salmonella</i> spp.	TSB	37 °C
<i>Escherichia coli</i>	TSB + 3% NaCl (w/v)	37 °C
<i>Vibrio</i> spp.	TSB + 3% NaCl (w/v)	35 – 37 °C

TSB, (Tryptic Soy Broth, Alpha Biosciences, Baltimore, MD)

NaCl, (Avantor, Center Valley, PA)

A single colony of each isolate was individually cultured in non-selective broth (Table 5.1) again, overnight (~ 12 – 14 hours). *Escherichia coli* and *Vibrio* spp. were inoculated in TSB with 3% NaCl (w/v) to simulate saline levels during the growth of sugar kelp to mimic the presence and growth of pathogens in kelp farming sites, and the possibility of contamination prior to harvesting (pre-harvest). The other pathogens were grown in broths without NaCl to simulate post-harvest contamination of sugar kelp from other sources. After the incubation period, broth cultures were centrifuged (Centrifuge 5810 R, Eppendorf, Hauppauge, NY) for 10 minutes at 5,000 x g. Pellets of each *Vibrio* spp. and *E. coli* broth culture were resuspended into 10 mL sterile imitation seawater (Imagitarium Pacific Ocean Water, Int. Pet Supplies & Distribution Inc, San Diego, USA), while pellets of *Listeria monocytogenes* and *Salmonella* spp. were resuspended into 10 mL autoclaved 0.75% saline (Difco, Sparks, MD) to achieve a 10x concentration of cells. Bacterial cultures of like species were diluted with either autoclaved seawater or 0.75% saline as appropriate to achieve the same concentration before mixing them to

serve as the stock culture for inoculation. Cultures were resuspended and diluted with either autoclaved seawater or 0.75% saline prior to inoculation to reduce environmental shock that could lead to death of some pathogen cells and enable pathogens to adapt to the salty conditions of sugar kelp. Two strains or species were used in this study and the diversity between the strains or species suggest a multiple introduction of pathogens to sugar kelp to replicate a real-time food contamination scenario from diverse sources and to ensure that at least one strain would survive on sugar kelp.

5.2.4 Microbial preparation and analysis

The prepared cocktail stock culture was diluted in order to deliver approximately 7 log CFU/mL and was inoculated (500 µL) onto each treatment of 25 g kelp blade or shredded slaw. A higher bacterial cell density was used (7 log CFU/mL) because a preliminary study with 5 log CFU/mL resulted in very low counts of 2.7 log CFU/g or below one hour post inoculation. To ensure even distribution of the cells, each inoculated kelp blade was shaken gently, and shredded kelp was mixed with a sterile rod for about 15 s inside the resealable zipper plastic bags. Inoculated samples in plastic bags were sealed and stored at the appropriate temperatures for further analysis.

After inoculation, samples were taken to determine levels of *Vibrio* spp., STEC, *Listeria monocytogenes*, and *Salmonella* spp. on the sugar kelp. Samples were hand homogenized with 225 mL selective broth (Table 5.2) for 2 minutes, before 1 mL aliquots were transferred for serial dilution with either autoclaved seawater or 0.75% saline (based on the bacterial culture) and subsequently plated on selective agar (Table 5.2). All plates were overlaid with 5 mL tempered (50°C Isotemp 105 water bath, Fischer Scientific, Dubuque, IA) soft Brain Heart Infusion (BHI) agar overlay prior to incubation

at optimal growth temperatures (Table 5.2). The overlay was prepared using Brain Heart Infusion Broth (Acumedia, Lansing, MI) with 0.6% Bacteriological Agar (Alpha Biosciences, Baltimore, MD). Treatments were processed in triplicate. Characteristic bacterial colonies from each plate were counted for enumeration of the surviving population.

For recovery of pathogens below the enumerable limit, a selective enrichment was conducted. The remaining the resealable zipper plastic bag containing the homogenized selective broth and the sample (after the removal of 1 mL aliquot for serial dilution), was incubated at optimum temperatures (Table 5.2) before plating on the same selective agar used for enumeration to determine the presence or absence of each pathogen. For enrichment of *Salmonella*, 0.1 ml of the incubated homogenate were subsequently transferred into 9 ml Rappaport-Vassiliadis broth and further incubated at prescribed conditions (Table 5.2) before plating on selective agar.

Table 5.2: Selective broth and agar, and incubation temperature used for microbial assessment

Pathogens	Selective broth for homogenization	Selective agar for enumeration	Incubation parameters for enrichment
<i>Listeria monocytogenes</i>	LEB ^a	PALCAM ^b agar 30 °C for 48 h	30 °C for 24 h
<i>Salmonella</i> spp.	BPW ^c	XLT-4 ^d agar 37 °C for 24 h	37 °C for 24 h RVB ^e : 41.5 °C for 24 h
STEC (<i>Escherichia coli</i>)	MBP-ACV ^f	TBX ^g agar 37 °C for 24 h	30 °C for 24 h
<i>Vibrio</i> spp.	APW ^h	TCBS ⁱ 35-37 °C for 48 h	30 °C for 24 h

^aLEB, (Listeria Enrichment Broth, Alpha Biosciences, Baltimore, MD)

^bPALCAM, (Polymyxin acriflavine lithium chloride ceftazidime aesculin mannitol, EMD Millipore Corporation, Billerica, MA)

^cBPW, (Buffered peptone water, Alpha Biosciences, Baltimore, MD)

^dXLT-4, (Xylose lysine tergitol-4, Sigma-Aldrich, St. Louis, MO)

^eRVB, (Rappaport-Vassiliadis broth, EMD Millipore Corporation, Billerica, MA)

^fMBP-ACV, (Modified buffered peptone, Neogen, Lansing, MI; with acriflavine-cefsulodin-vancomycin, Himedia Laboratories, Mumbai, India)

^gTBX, (Chromocult Tryptone Bile X-Glucuronide, EMD Millipore Corporation, Billerica, MA)

^hAPW, (Alkaline peptone water, Oxoid Ltd, Hants, UK)

ⁱTCBS, (Thiosulfate-citrate-bile salts-sucrose, BD Difco, Sparks, MD)

5.2.5 Storage conditions

Inoculated samples were placed at ambient temperature (22 °C) in a biological safety cabinet, refrigerator (4 °C) and a cooling incubator (10 °C) for the prescribed time before microbial analyses.

5.2.6 Statistical analysis

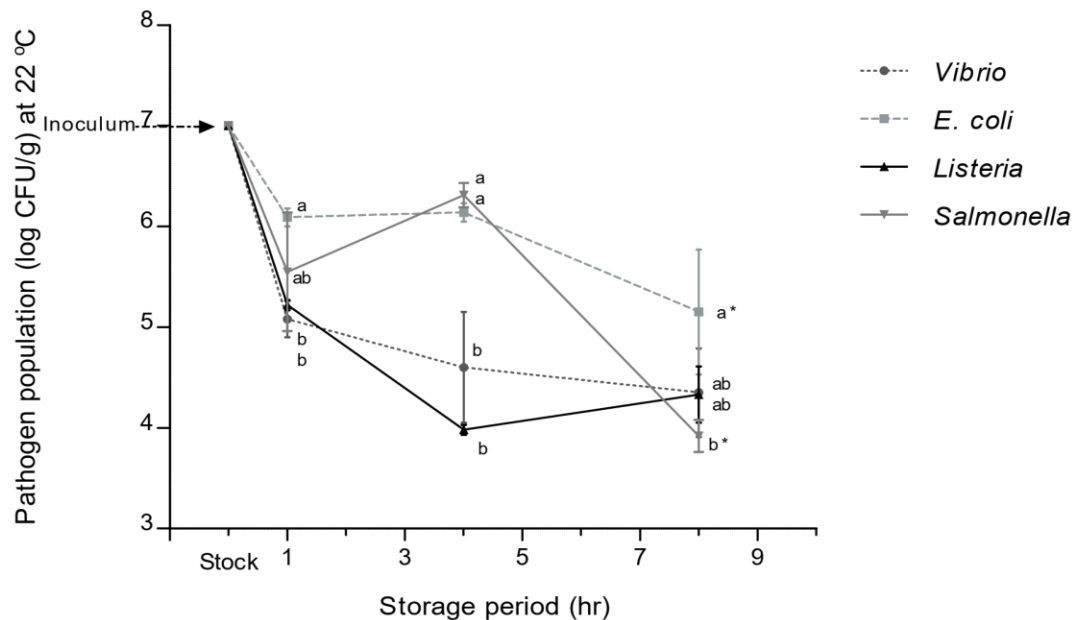
The results were calculated as mean of three replicates \pm standard deviation (SD). Tukey's HSD test was used (SPSS version 20; IBM, Armonk, NY, USA) to analyze the significant mean separation ($P \leq 0.05$) between each bacterial log reduction at 4, 10, and 22 °C. The linear regression model was used to compare log reductions among treatments for each pathogen.

5.3 Results and discussion

5.3.1 Effect of temperature on inoculated pathogens

All foods are ecosystems comprised of intrinsic and extrinsic factors. The intrinsic factors are inherent to the food including pH, water activity, and nutrients, and the extrinsic factors are external to it (temperature and gaseous environment) (Montville and Matthews 2013). All these factors influence microbial growth and survival in food, causing the growth or injury of microbes, or making microbes unculturable. Fresh produce is susceptible to bacterial pathogen contamination, with particularly leafy green vegetables, responsible for a high number of reported foodborne illness cases (228 out of 1797) in the U.S. from 2010 to 2017 (CDC, 2017). Fresh sugar kelp has a high water activity (Sappati et al. 2019), good amount of proteins (Stévant et al. 2017), high polysaccharide content and a fair amount of lipids (~5-20% dw) (Stévant et al. 2017; Imchen 2021), and these can facilitate microbial survival and growth, thus, the influence of temperature on microbial growth cannot be overstated. According to Sant'Ana et al. (2012), inappropriate storage temperature has been reported as one of the three most important faults contributing to the occurrence of outbreaks due to consumption of salads. The free water in foods that is utilized by microbes is mostly unavailable in frozen states, leading to an unfavorable condition that minimizes bacteria growth. Whereas retarded microbial growth has been reported in food at refrigerated temperatures (Söderqvist et al. 2017). Microbial cells grown at refrigerated temperature express different genes and are physiologically different from those grown at ambient temperature (Montville and Matthews 2013), influencing their growth kinetics. Therefore, storage temperature (refrigerated or ambient) can have a significant impact on

the two pathogenic psychrophiles (*Vibrio* and *Listeria*) and mesophiles (*Escherichia coli* and *Salmonella*) utilized in this study. In our study, the overall log reduction of all four pathogens after storage at ambient temperature was low, ranging from 0.69 – 3.08 log CFU/g (Figure 5.1) as compared to 0.88 – 5.20 log CFU/g (Figure 5.2) and 1.42 – 5.30 log CFU/g (Figure 5.3) for 10 °C and 4 °C, respectively. These results suggest that an increase in temperature will favor the growth of bacterial pathogens and their survival, necessitating extra postharvest practices such as blanching (Appendix K) within the seaweed supply chain to ensure the safety of the products.



Error bars represent standard deviation.

Small letters denote significant differences between four pathogens, within time point.

Asterisks denote significant difference between population at the end of storage and at time 1 hr.

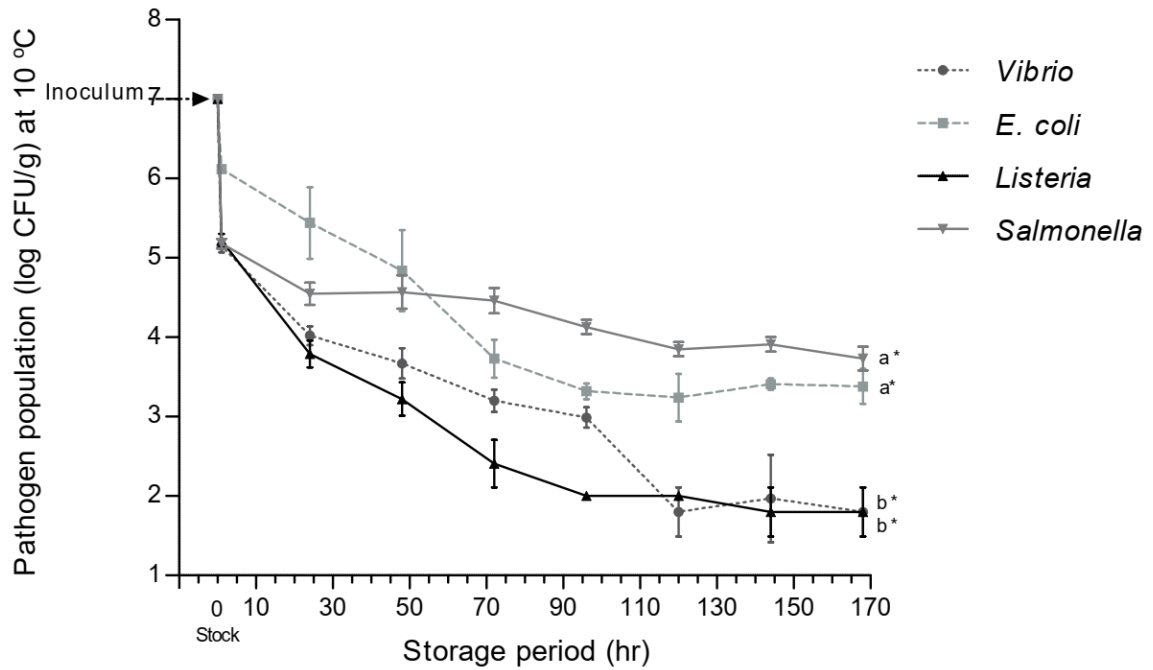
Figure 5.1: Mean population of four bacterial pathogens inoculated on sugar kelp (both whole blade and shredded slaw) and stored at ambient temperature (22 °C), n = 6

Figure 5.1 shows a lesser log reduction in *Escherichia coli* (STEC) and more gradual reduction in *Salmonella* sp. populations at ambient temperature, confirming mesophiles surviving better than the two psychrophiles (*Vibrio* and *Listeria*) inoculated on the sugar kelp at 1 and 4 hr storage time. Although *Salmonella* and STEC optimally

grow at 37°C, they survive in a wider range of temperatures in food (Doyle and Buchanan 2013). After the 8 hr ambient storage, log reduction for *Salmonella* (3.08 ± 0.15) was significantly higher than for STEC (1.85 ± 0.62) but not *Vibrio* (2.65 ± 0.44) or *Listeria* (2.67 ± 0.28). These results suggest that *Vibrio*, *Listeria* and especially, STEC may survive very well when harvested sugar kelp are not immediately subjected to lower temperature storage. Interestingly, the two psychrophiles were not significantly different from the mesophiles at the end of ambient storage, confirming other studies where an increase in temperatures positively correlated with the growth of some psychrophiles like *Vibrio* (Mahmud et al. 2008; Montville and Matthews 2013). These psychrophiles may contaminate sugar kelp before harvesting and if that happens, there is a high probability of survival during postharvest ambient storage as compared to storage at lower temperatures. Therefore, seaweed processors should consider processing and storing sugar kelp at lower temperatures to minimize pathogen growth and/or survival.

Many foods are chilled and kept refrigerated during storage and retailing, after harvesting or processing. These refrigerated temperatures during storage prevent growth of microorganisms that survive processing (Yousef and Balasubramaniam 2013). The trends of log reduction in all four inoculated pathogens in sugar kelp stored at 4 °C and 10 °C indicate the impact of low temperatures on bacterial growth. The lower temperature (4 °C) generally resulted in higher log reduction in all four pathogens during and at the end of storage than the relatively higher temperature (10 °C) (Figure 5.2 and 5.3), which could be termed as an abuse of refrigerated temperature. Since recommended refrigerated temperature by U.S. FDA is 4 °C and maximum refrigerated temperature in Sweden for perishable foods including leafy vegetables is 8 °C (Møller et al. 2016; FDA

2021). Among the four pathogens, an abuse in temperature resulted in significantly better survival of STEC populations (Figure 5.5).

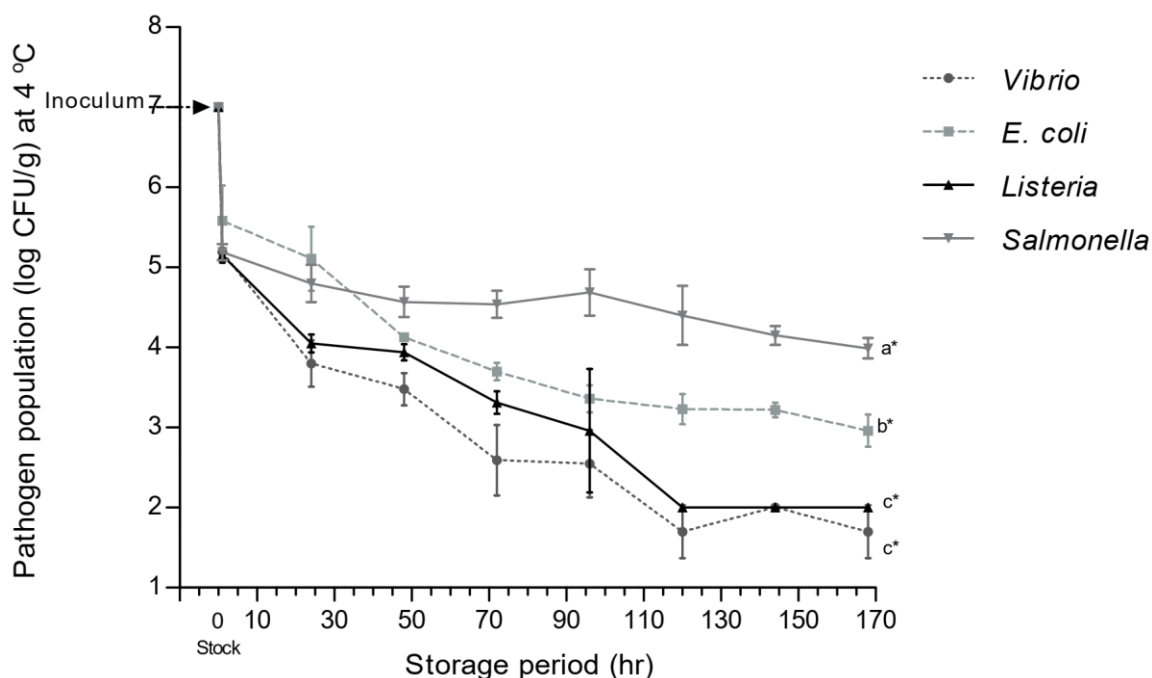


Error bars represent standard deviation.

Small letters denote significant differences between four pathogens, within time point.

Asterisks denote significant difference between population at the end of storage and at time 1 hr.

Figure 5.2: Mean population of four bacterial pathogens inoculated on sugar kelp (both whole blade and shredded slaw) and stored at 10 °C (n = 6)



Error bars represent standard deviation.

Small letters denote significant differences between four pathogens, within time point.

Asterisks denote significant difference between population at the end of storage and at time 1 hr.

Figure 5.3: Mean population of four bacterial pathogens inoculated on sugar kelp (both whole blade and shredded slaw) and stored at 4 °C (n = 6)

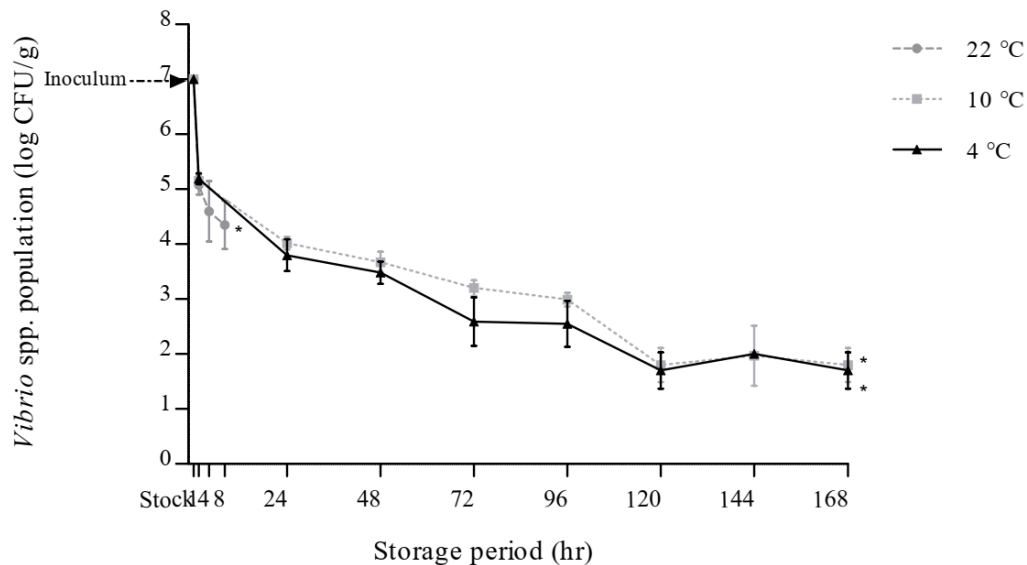
Our previous study failed to detect any of these four pathogens in uninoculated, commercially harvested sugar kelp in Maine (Chapter 2 and 3; Akomea-Frempong et al. 2021a, b). However, these results do not guarantee the safety of sugar kelp entirely, since *Vibrio* spp. and *Escherichia coli* were detected in sugar kelp grown at unauthorized sites for shellfish in Maine (Barberi et al. 2019). Therefore, the present study used *Vibrio* spp. and *E. coli* to simulate the possibility of preharvest contamination since these pathogens are increasingly becoming prevalent in growing environments of kelp (Mahmud et al. 2008; Wyness et al. 2019). *Listeria* and *Salmonella* were employed in our study design to create a postharvest contamination simulation as these pathogens are commonly found on processing units or contaminate food during postharvest practices. So, the storage study

of inoculated sugar kelp at these temperatures was conducted for a realistic evaluation of the survival of these pathogens if commercially sold sugar kelp were to get contaminated and be subjected to different storage temperatures at home or retail. Results are important for producers to consider using approved production sites for seaweed production to ensure low risk of preharvest contamination and implement interventions including adherence to appropriate storage temperatures to mitigate food safety risks from postharvest contamination of sugar kelp.

There was a significant decrease in population after 7 days of storage in all four pathogens on kelp stored at 4 °C and 10 °C as compared to time 0 (1 hr). These results suggest that temperature and other factors including pH and food matrix of the sugar kelp among others may have contributed to the decrease in bacterial pathogen population. Unpredictably, the bacterial population significantly decreased ($P \leq 0.05$) in the two psychrophiles to a greater extent than in the two mesophiles stored at 4 °C and 10 °C (Figure 5.2 and 5.3). Although psychrotrophs such as *Vibrio parahaemolyticus* grow and proliferate in fluctuating cold saline environment, results from our study signify the importance of other bacterial growth factors aside from temperature for growth of pathogens on sugar kelp. The conditions to favor bacterial growth may include the synergistic effect of temperature, water activity and some intrinsic factors such as secondary metabolites including bromoform (Paul et al. 2006) in kelp (Kuyper et al. 2018) that have antimicrobial properties against some pathogens. Graham et al. (2016) reported high amounts of polysaccharides and phlorotannin in kelp and these compounds have antimicrobial effects against *Escherichia coli* and *Vibrio* sp., respectively (Cabral et al. 2021).

5.3.2 Survival of inoculated *Vibrio* spp. on sugar kelp

This study was conducted to see if product form, temperature and storage time had a significant influence on the survival of *Vibrio* spp. inoculated on sugar kelp. To be specific, shredding or cutting sugar kelp may be a point of bacterial contamination for the sugar kelp value chain. Moreover, shredding kelp may expose bacteria to readily available nutrients in the product. Thus, we hypothesized that product form (shredded slaw), higher storage temperature and a longer storage time may increase *Vibrio* spp. population or reduce the log reduction of *Vibrio* spp. inoculated on sugar kelp. Results (Appendix K) show that 90.8% of the variance in *Vibrio* spp. log reduction ($F = 372.8$, $P \leq 0.05$) can be accounted for by product form, temperature, and storage time (predictors). However, from the coefficient table (Appendix K), only storage time had a significant impact ($P \leq 0.05$) on log reduction of *Vibrio* spp. and could be used to predict *Vibrio* survival as compared to storage temperature and product form.



Error bars represent standard deviation.

Asterisks denote significant difference between population at the end of storage and at time 1 hr.

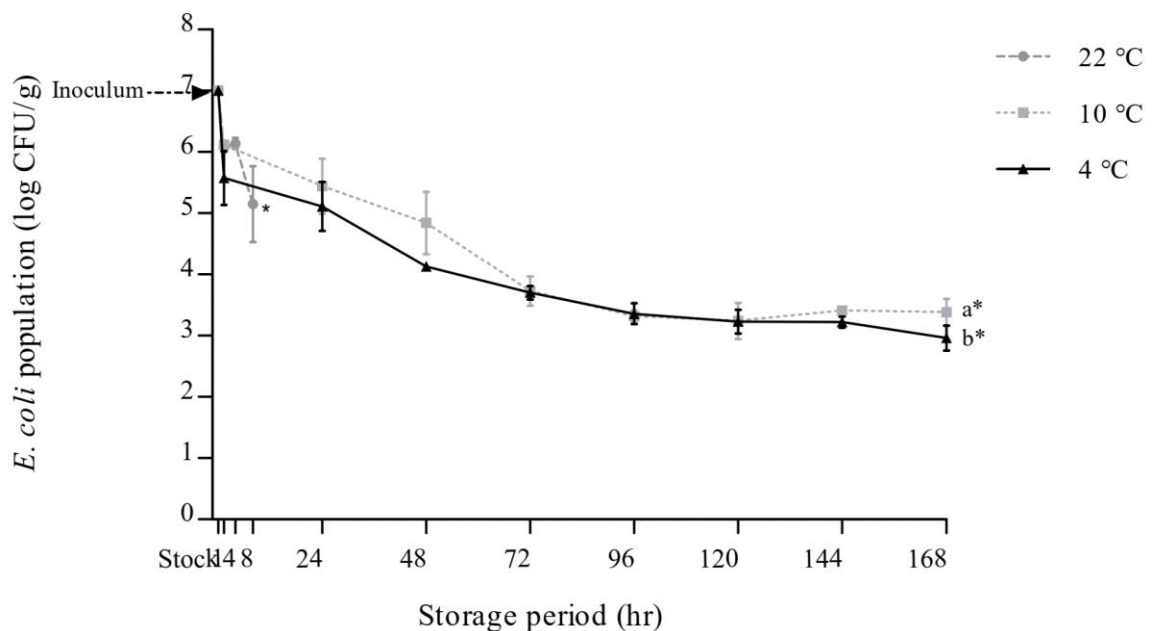
Figure 5.4: Mean population of *Vibrio* spp. inoculated on sugar kelp (both whole blade and shredded slaw) subjected to various storage temperatures and time (n = 6)

Our study used a cocktail of *Vibrio vulnificus*, which increased in population on coastal seaweed harvested in Korea as water temperatures increased during a season-long evaluation (Mahmud et al. 2007), and *V. parahaemolyticus*, known to be psychrotrophic (Marth 1998). However, the increase in temperature from 4 °C to 10 °C (signifying an abuse in temperature) did not have a significant impact on log reduction during storage. A significant decrease in population was recorded after storage (day 7) as compared to an hour post inoculation of *Vibrio* spp. in sugar kelp stored at both 4 °C and 10 °C (Figure 5.4). In addition, the gradual increase in log reduction may be due to breakdown and availability of secondary metabolites, polysaccharides, phlorotannins and bromophenols in sugar kelp that have recently gained attention as potential antimicrobials (Cabral et al. 2021). The gradual decrease in *Vibrio* population during storage suggest that a longer refrigeration time had an impact on the survival of *Vibrio*. But storing kelp more than a week may not be ideal for consumption as sensory evaluation of refrigerated kelp (2 °C and 7 °C) reported a reduced overall quality score, from ~13 to below 5 at day 7 of storage, using a 15 cm unstructured line scale (Nayyar 2016). The product form did not have any significant effect on *Vibrio* spp. log reduction, and this may have been due to polysaccharide that oozed from both product forms of kelp. The increasing prevalence of *Vibrio* spp. in the U.S. due to an increase in water temperatures and the production of cultivated seaweed in marine ecosystems intensifies the need for control strategies in processing fresh seaweed in the U.S. It is important to note that *Vibrio* populations significantly decreased from ~ 5 log CFU/g (time 0) to ~ 1.7 log CFU/g (day 7), which is a positive outcome for seaweed processors in storing sugar kelp at lower temperatures since the infectious dose for *V. parahaemolyticus* is ~10⁶ cells (Oliver et al. 2013). This

may also suggest need for more investigation as *Vibrio* species can exhibit a viable but not culturable state at lower temperatures (Oliver et al. 2013).

5.3.3 Survival of inoculated shigatoxigenic *Escherichia coli* (STEC) on sugar kelp

Data show a significant effect ($F = 239.4$, $P \leq 0.05$) of log reduction for *E. coli* with $R^2 = 0.867$, suggesting that 86.7% of the variation is predicted by product form, storage temperature and storage time. From the coefficient table (Appendix K), storage time and temperature had a significant impact ($P \leq 0.05$) on log reduction of STEC and could be used to predict STEC survival as compared to product form.



Error bars represent standard deviation.

Small letters denote significant differences at the end of storage between 4 °C and 10 °C.

Asterisks denote significant difference between population at the end of storage and at time 1 hr.

Figure 5.5: Mean population of STEC inoculated on sugar kelp (both whole blade and shredded slaw) subjected to various storage temperatures and time (n = 6)

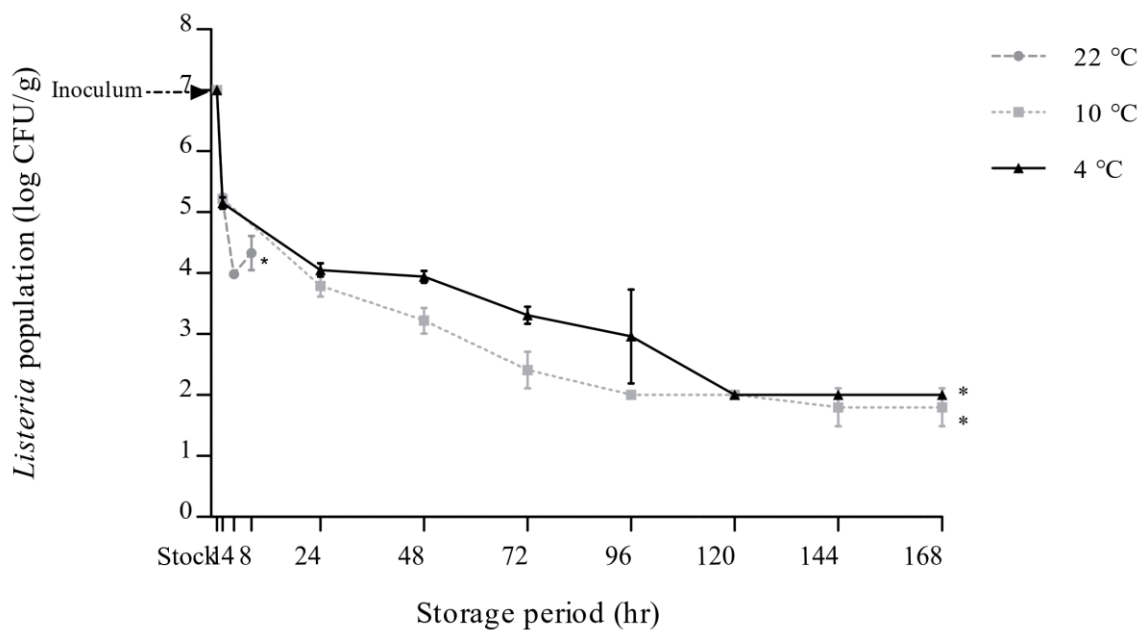
There was less than a 2-log reduction of STEC after 8 hours at 22 °C, while 10 °C recorded about 3.6-log reduction and ~4-log reduction for 4 °C after seven days of storage (Figure 5.5). It is important to note that an abuse of temperature (10 °C) during

refrigeration may lead to a significantly higher surviving STEC population as indicated by the significantly lower log reduction of STEC at 10 °C as compared to 4°C after 7 days storage. When STEC was inoculated on shredded lettuce and sliced cucumber that were air packed and stored at 5 °C, 12 °C and 21 °C for 14 days, Abdul-Raouf et al. (1993) reported about a 1 log reduction of STEC population at 5 °C and an increase in population at 12 °C and 21 °C on day 7 and after storage (14 days). The results were quite different from our study as there were significant log reductions of STEC in sugar kelp after storage at all the three temperatures (Figure 5.5). Although STEC are normally associated with leafy vegetables because of the addition of manure, which may be contaminated, seaweed could also be contaminated with STEC from run-off water from farms and municipalities into water bodies where they are cultivated. Also, sugar kelp may be contaminated if not processed in hygienic facilities as this is another potential source of STEC contamination (Luna-Guevara et al. 2019). Although STEC contamination on kelp may be rare, the high STEC population after day 7 storage is concerning to seaweed processors as the infectious dose for STEC to cause illness is as little as 10 cells (Li et al. 2013).

5.3.4 Survival of inoculated *Listeria monocytogenes* on sugar kelp

Log reduction for *Listeria monocytogenes* was significant ($F = 334.7$, $P \leq 0.05$) with $R^2 = 0.901$, suggesting that 90.1% of the variation is predicted by product form, storage temperature and storage time. From the coefficient table (Appendix K), storage time and temperature had a significant impact on *L. monocytogenes* log reduction, but product form did not. This could be due to the unavailability of adequate nutrients on the part of whole blades and the availability of abundant polysaccharides in shredded kelp

having a bacteriostatic effect on *L. monocytogenes*, since these polysaccharides are reported to have antimicrobial properties that inhibit growth of microorganisms (Cabral et al. 2021). A previous study reported that 10 µg extract of fucoidan (polysaccharide) found in seaweed (*Sargassum swartzii*) inhibited *Staphylococcus aureus* (9 ± 0.67 mm inhibition), *Proteus vulgaris* (7 ± 0.72 mm inhibition) and *Escherichia coli* (15 ± 0.28 mm inhibition) (Vijayabaskar et al. 2012).



Error bars represent standard deviation.

Asterisks denote significant difference between population at the end of storage and at time 1 hr.

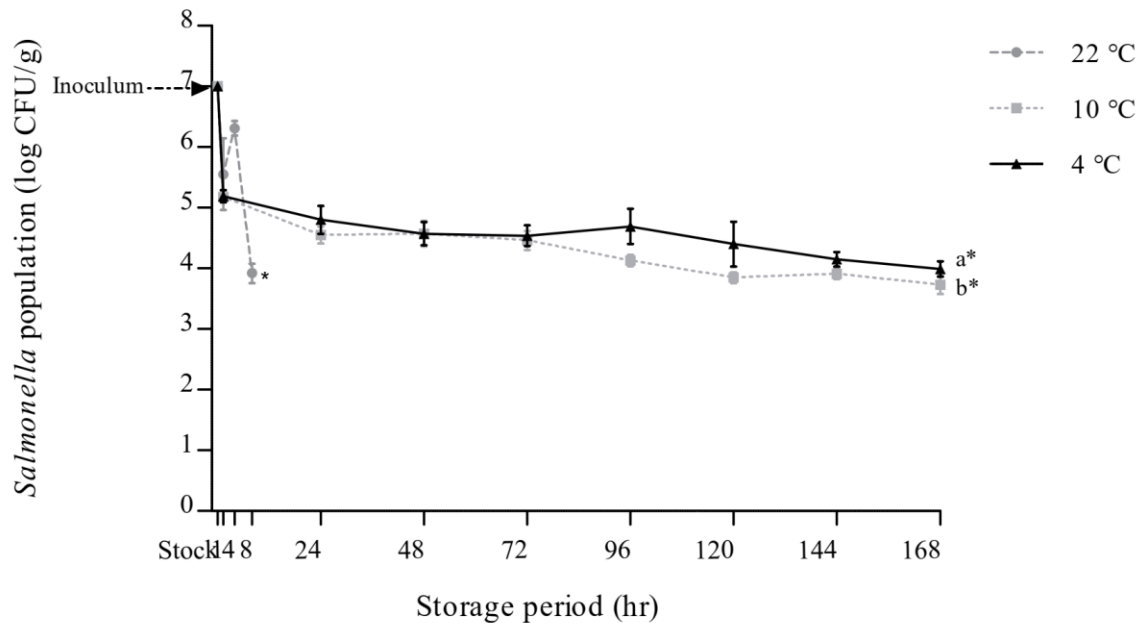
Figure 5.6: Mean population of *L. monocytogenes* inoculated on sugar kelp (both whole blade and shredded slaw) subjected to various storage temperatures and time (n = 6)

The immediate decline in *Listeria monocytogenes* population one hour post inoculation and even the slight increase at 8 hr when stored at 22 °C can suggest a short lag phase in this study (Figure 5.6) as compared to other studies, and warrants investigation of population dynamics when stored at ambient temperature for longer duration. When *Listeria* spp. was inoculated on apple (Kim et al. 2018) and cheese

(Hassanien et al. 2014), it took days for pathogens to grow in the respective food without preservatives. The short lag phase could be because *L. monocytogenes* was resuspended in seawater prior to inoculation onto sugar kelp to reduce environmental stress. Previous studies on the growth and survival of *L. monocytogenes* on spinach leaves provided quite different results as compared to our study. There was about 1.2 to 2.3 log-increase of *L. monocytogenes* inoculated on white cabbage, leek, kale, red chard and parsley after 10 days storage at 7 °C (Lokerse et al. 2016). Additionally, there was a 0.4 log reduction in *L. monocytogenes* on spinach after storage in the same study and the decrease was speculated to be as a result of antimicrobial compounds in spinach. There was an increase in *L. monocytogenes* on baby spinach after 3 days but populations decreased at the end of a 7-day storage at both 8 °C and 15 °C (Söderqvist et al. 2017). Similarly, Culliney and Schmalenberger (2020) reported an increase in *L. monocytogenes* (1.08-2.66 log CFU/g) on spinach, rocket and lettuce during a shelf-life challenge study where the ready-to-eat vegetables were stored at 8 °C for 9 days. However, the higher log reduction in this study (1.78-5.20 log CFU/g) suggests the effect of other factors aside from temperature influencing bacterial growth. These include bacteriostatic compounds in seaweed (Cabral et al. 2021), pH and water activity that were not included in our study design. Survival of *Listeria* is of concern irrespective of the storage temperature as the infectious dose of *Listeria* is 100 cells (Ryser and Buchanan 2013) and voluntary guidance has been provided to the U.S. industry to help meet legal target of less than 1 cell per 25 g of ready-to-eat food (FDA 2017).

5.3.4 Survival of inoculated *Salmonella* on sugar kelp

Results (Appendix J) show that 55.5% of the variance in *Salmonella* spp. log reduction ($F=45.7$, $P\leq 0.05$) can be accounted for by the product form, storage temperature and time. Only storage time had a significant impact on *Salmonella* spp. log reduction and could be used to predict *Salmonella* survival as compared to storage temperature and product form (Appendix K). From the model, temperature is not a significant predictor for *Salmonella* survival and this confirms the wide range of growth ($5\text{ }^{\circ}\text{C} - 47\text{ }^{\circ}\text{C}$) for *Salmonella* (D'Aoust 1989; Li et al. 2013).



Error bars represent standard deviation.

Small letters denote significant differences at the end of storage between $4\text{ }^{\circ}\text{C}$ and $10\text{ }^{\circ}\text{C}$.

Asterisks denote significant difference between population at the end of storage and at time 1 hr.

Figure 5.7: Mean population of *Salmonella* spp. inoculated on sugar kelp (both whole blade and shredded slaw) subjected to various storage temperatures and time ($n = 6$)

There was about a 3-log reduction of *Salmonella* spp. in sugar kelp samples stored at $22\text{ }^{\circ}\text{C}$ after 8 hr, $10\text{ }^{\circ}\text{C}$ and $4\text{ }^{\circ}\text{C}$ on day 7 (Figure 5.5). Overall, temperature had no

significant impact on log reduction but considering samples after the storage time, *Salmonella* spp. were significantly higher in population at 4 °C as compared to samples stored at 10 °C (Figure 5.7). This could be that 4 °C may serve as an adverse condition for *Salmonella* spp. (mesophile) and bacterial cells may have expressed cold shock genes to reduce the rate of population growth (Montville and Matthews 2013) as compared to 10 °C or 22 °C, where bacterial cell were exposed to several bacteriostatic compounds of seaweed during the break down of the sugar kelp food matrix. It is important to adhere to practices that may minimize risk of *Salmonella* contamination since storing of kelp at refrigerated temperatures may not be enough to mitigate growth. Comparing our study to other vegetable storage studies, *Salmonella* increased (~2-3 log CFU/g) as storage time progressed in whole and sliced cucumber stored at 23 °C (4 days storage time) and recorded some significant log reductions (~0.7-2.3) in whole and sliced cucumber stored at 4 °C after 21 days (Bardsley et al. 2019). The slightly higher log reduction (3.01-3.27) in our study emphasizes that high salt content in sugar kelp may have significant impact on bacterial survival by altering the water activity of the product. Moreover, it emphasizes the importance of pH change since *Salmonella* produces acids during growth, and the availability of several bacteriostatic compounds in sugar kelp against the growth of *Salmonella* spp. The increase in *Salmonella* population at 4 hr suggest the need to immediately cool products since a 4 hr storage of kelp at ambient temperature may be enough to facilitate bacterial growth. Moreover, our study suggests that *Salmonella* survived on refrigerated kelp to a greater extent than any other pathogen tested.

5.4 Conclusions

The present study shows that different storage temperatures had different effects on the survival of *Vibrio* spp., STEC, *Listeria monocytogenes* and *Salmonella* sp. inoculated on sugar kelp. The differences in pathogen populations denote the significance of storage temperatures, and other factors which were not evaluated in this study including pH, water activity, salinity, and product matrix (antimicrobial properties) can further augment their risk. Regarding the short shelf-life of refrigerated sugar kelp, the survival of these four bacterial pathogens on both whole and shredded sugar kelp highlights the need to reduce the likelihood of contamination events throughout the sugar kelp supply, since their populations after storage were above infectious doses except for *Vibrio* sp. Results emphasize the need for strict adherence to temperature control for sugar kelp after harvesting and underlines that temperature abuse may support pathogen survival, or even growth in sugar kelp. Specifically, a longer storage period at ambient temperature can support *Vibrio*, *Listeria* and particularly STEC growth more than *Salmonella*, contrary to the high *Salmonella* population observed at lower temperatures. The lower populations recorded in the two preharvest pathogens (*Vibrio* and STEC) as compared to *Salmonella* when stored at 4 °C or 10 °C is encouraging as postharvest pathogen contamination can be largely minimized by strict adherence to sanitation standard operating procedures (SSOP) as compared to preharvest pathogen contaminations. An abuse in refrigeration temperature from 4 °C to 10 °C can favor a better STEC survival in seaweed. However, extra measures are necessary such as implementing a “kill step” in the processing of fresh sugar kelp to ensure safety of the product. This is because *Vibrio* (preharvest pathogen and psychrophile) populations were

low at lower temperatures, but they could be viable as they can exhibit a viable but non-culturable state. We recommend that considerable attention be paid by the seaweed industry to minimize the contamination of sugar kelp with both pre- and postharvest pathogens examined in this study especially STEC and *Salmonella*, because they can be of significance to the public health, especially among consumers who prefer raw unprocessed seaweed.

CHAPTER 6

OVERALL CONCLUSIONS AND RECOMMENDATIONS

Postharvest loss is a kind of food loss and waste that occurs at different stages of a food value chain after harvesting, including processing, storage, distribution, retail, and consumption. In the United States, about one-third of all available food goes uneaten through loss or waste for many reasons, with some types of loss such as food spoilage occurring at every stage of the production and supply chain. Unfortunately, these spoiled foods have great impact on economic value and profits of industries. Additionally, food contamination contributes to postharvest losses and can cause situations of potential public-health significance, which also represent significant economic losses to consumers and producers. So, there is an urgent need for postharvest practices to extend the shelf-life of food, maintain food safety and add value to food products, especially those that are emerging on the U.S. food market such as seaweed, to minimize food loss.

There are several challenges associated with the nascent edible farmed seaweed industry in the U.S. Fresh seaweeds including sugar kelp have short shelf-life, thus, applying preservation methods such as drying, freezing, minimal processes (e.g. blanching), and fermentation to extend the shelf-life and yield high quality products is important in reducing postharvest loss.

There are several postharvest practices employed by seaweed processors that may also affect seaweed quality and safety, and may contribute to food loss. For example, shredding of kelp because of its intended use as kelp “noodles” may affect kelp quality as compared to using the intact whole blade. Seaweeds are either vacuum packaged or not

prior to processing or storage, and they are either blanched or not prior to freezing or drying, all of which have some associated impacts on kelp quality and safety. Also, because seaweeds are predominantly dried, they are normally rehydrated prior to consumption or processing and these rehydration regimens may affect kelp qualities and safety.

These studies were conducted to evaluate and recommend rehydration procedures and minimal processes such as pre-freezing blanching that can yield high quality kelp and kelp products. Additional objectives were to evaluate the use of minimally processed sugar kelp as raw material for developing consistent high quality food products independent of harvest season, and to assess the potential impacts of different storage temperatures on the survival of bacterial pathogens on kelp.

Sugar kelp is harvested in spring to early summer in Maine, thus, a one-year frozen study was important to assess the qualities of the frozen kelp between harvesting seasons in order to increase the availability of fresh-like kelp throughout the year. Results confirmed that pre-freezing practices impacted frozen kelp quality. Product form affected texture significantly, as shredded kelp had fairly consistent hardness values during frozen storage as compared to whole blades. Although direct comparison is not possible due to the different texture methods used for the two product forms, shredded kelp yielded lower hardness values throughout frozen storage as compared to whole blade. Developing a robust method that can measure the texture of both product forms will facilitate direct comparison that will help processors to choose the appropriate product form either to maintain the textural quality of kelp or to provide convenience in handling kelp.

Blanching prior to frozen storage produced high quality frozen kelp for at least six months as compared to unblanched frozen kelp. Frozen storage of unblanched kelp resulted in increased fungi count, reduced hardness and significant discoloration, specifically reduced lightness (L^* value) and greenness (increasing a^* value) during frozen storage compared to blanched kelp irrespective of the product form. Results revealed that consumers may consider minimal processing such as blanching prior to freezing to enjoy quality fresh-like seaweed throughout the year. This study did not evaluate seaweed qualities post-blanching prior to freezing and we recommend that such analyses be done to differentiate the impacts of blanching from those of frozen storage.

This study was the first report to compare multiple blanching procedures used by seaweed processors including high (100 °C) and low (80 °C) blanching temperatures, longer (30 s) and shorter (5 s) blanching times, and direct-immersion (DI) versus vacuum packaged blanching (VP) to preserve kelp during one-year of frozen storage (-20 °C). Blanching kelp with the VP method, blanching at 100 °C and for 30 s resulted in high moisture content, total phenolic content, ferric reducing antioxidant power values, and high hardness and chewiness values. These results indicate that the VP blanching method can yield high quality kelp during frozen storage in addition to providing convenience during processing and handling. However, care should be taken in choosing appropriate plastic bags and during vacuum sealing to prevent leaching of unwanted chemicals into food products and to inhibit the growth of obligate anaerobic food pathogens, respectively. Studying the effects of additional variables such as freezing temperatures may further increase the potential profits of the sugar kelp industry and the consistency in producing high quality sugar kelp. Measuring the internal temperature of the vacuum

packed pouches during blanching will be a significant step in standardizing these blanching procedures for the seaweed industry, especially processors that may consider vacuum packaged blanching method aside the common blanching method (direct immersion) among seaweed processors in Maine.

Several kelp qualities were evaluated including its microflora and drip loss, to address the shelf-life of kelp in a broader view. We observed that changes in drip loss affected texture over frozen storage time, thus quantifying the drip loss and calculating the percent softening over time provided crucial information about quality loss. Evaluating antioxidant activity during storage with additional methods such as oxygen radical absorbance capacity (ORAC) or 2,2-diphenyl-1-picrylhydrazyl (DPPH) may have given a more comprehensive account of antioxidant capacity during storage as compared to using only the FRAP method at the end of storage. Also, evaluating the impact of pre-freezing blanching procedures and frozen storage on the physicochemical and microbial qualities of sugar kelp harvested at different timepoints in the season (e.g. late March vs. late May) may help kelp producers and processors to maximize frozen kelp qualities irrespective of the harvest time. This suggestion is based on a prior study that reported great variation in kelp quality harvested at two different seasons (Schiener et al. 2014). This information will help processors to develop appropriate procedures prior to and during storage, and provide a strong foundation to optimize minimal processing in light of preserving fresh sugar kelp via freezing.

In the second study, we evaluated the impacts of blanching, freezing, and fermentation on kelp quality and consumer acceptability in two experiments. The objective of the first experiment was to determine the impact of blanching (100 °C) for 1

and 3 min on kelp quality and consumer acceptance when developed into kelp salad as compared to raw kelp. Blanching treatments were significantly lighter (higher L^* value) and greener (lower a^* value), and had reduced instrumental hardness. Blanching treatments increased consumer liking scores for color, flavor and overall product liking when kelp was formulated into salad as compared to raw kelp salad. These results suggest that blanching can help increase consumer liking for sugar kelp, making blanching important not only as a pretreatment for long-term preservation methods such as freezing and drying. These results are important for kelp processors and for food research and development scientists to consider when developing kelp products for the market.

The results from the second experiment indicate that a safe and high-quality sauerkraut can be prepared from sugar kelp even when subjected to blanching and freezing, when produced under good manufacturing and sanitation practices. Kelp was blanched (100 °C) and/or frozen (-20 °C) prior to mixing with cabbage and fermenting into sauerkraut. Blanched treatments were significantly lower in instrumental hardness but higher in brightness and greenness when compared to raw treatments, which could influence consumer acceptability.

Sensory evaluation of kelp sauerkraut can help accelerate the potential market opportunities of these products. Therefore, blanched treatments (blanched and blanched frozen) were selected based on consumer scores from the kelp salad experiment, alongside a 100% cabbage sauerkraut for sensory evaluation in this experiment. Interestingly, consumers liked the harder cabbage sauerkraut to a greater extent than the less hard blanched kelp sauerkraut. In contrast, high consumer liking was observed for the less hard kelp in blanched kelp salad compared to the harder raw kelp salad. We

recommend that kelp processors consider the end product and consumer preference prior to blanching or optimize the blanching process to overcome texture challenges when developing kelp products.

Furthermore, freezing kelp after blanching appeared to result in adequate quality characteristics since consumer liking of blanched-frozen kelp sauerkraut was not different from the blanched kelp sauerkraut. This is promising, since freezing masked some undesired flavors in kelp, can extend kelp shelf-life and supply, and did not have significant impact on consumer acceptability. However, the impact of long term frozen storage of kelp on consumer acceptability should be assessed and will be more informative to the kelp industry as kelp in this study was frozen only for 24 hr.

After sauerkraut fermentation, *Vibrio* spp., *Listeria monocytogenes*, *Salmonella* spp., and *Staphylococcus aureus* were not detected and there was no difference in levels of spoilage microorganisms for all treatments, although a presumptive *Vibrio* was detected prior to fermentation in the raw kelp treatment. This validates that fermentation inhibits pathogen growth and all treatments did not appear to influence the growth of spoilage microorganisms in sauerkraut. Optimizing the fermentation process and the use of different freezing parameters could provide useful information for other researchers developing sauerkraut from kelp. While the results from this study provide a strong foundation for the quality assessments of fermented seaweed subjected to various minimal processes, studying the effects of these processes on chemical constituents and nutrients in seaweed will help optimize the processes to maximize the levels of certain nutrients.

Most seaweeds, in particular kelp, are dried to extend their shelf-life, with sun-drying being the most common process around the world. Sun drying poses some challenges including exposure of seaweed to UV light, and may be time consuming. Other drying methods including hot air convective drying are also used in Maine to increase seaweed drying capacity during times of limited sunshine and to retain nutrient profile of the dried product (Sappati et al. 2019). Mostly, these dried seaweeds are rehydrated before consumption. The third study was conducted to determine the effects of rehydration temperature (22, 75 and 100 °C) on the physicochemical properties and microbial quality of sugar kelp. Understanding the rehydration kinetics of dried kelp and its impact on kelp quality can help optimize rehydration regimes to attain high quality rehydrated kelp product. In the study, the drying of raw sugar kelp samples were processed in three separate groups with the same drying parameters to represent a replicate for rehydration. The raw materials were mixed together prior to the groupings, thus each group was a representative of the starting material.

Overall, rehydration ratios were similar among the rehydration temperatures. A quantification of rehydration rates was not included in our study, which may have better explained the rehydration kinetics. Most rehydration rates are usually estimated by experimental data fitted to empirical models including the Weibull, Peleg's and first-order rather than the analytical approach used in our study. The empirical approach represents pure kinetics of the physical processes and helps define rehydration constants and how independent variables correlate with each other.

The literature on the physicochemical impact of rehydration of dried seaweed is scarce. In our study rehydration increased greenness and lightness of kelp, which may

positively affect consumer acceptability. Also, rehydration reduced iodine content in sugar kelp as compared to dried and raw samples, which is very promising for consumers having concerns about the high iodine levels in sugar kelp. As seaweed producers create diverse products made with dried seaweed, standardization of rehydration procedures for high quality products is needed. The study used initial water temperatures of 100 °C, 75 °C and 22 °C, which dropped rapidly during rehydration. Thus the need for optimizing the rehydration process with various seaweed species and seasonal dried seaweed product for both the industry and consumers at home to increase marketability. The lessons learned from this work will serve as a groundwork for future research in this area.

We recommend that rehydration of seaweed should also focus on other nutritional constituents and chemical compounds to give a wholistic approach to the seaweed industry in making seaweed a superfood. Moreover, understanding the microscopic movement of moisture inside kelp during rehydration, especially when subjected to different temperatures will help determine heat and mass transfer rates to better predict rehydration rates of seaweed. Also, consumer acceptability of these rehydrated products is worth investigating to develop nutritious food that are liked by U.S. consumers.

In the absence of good manufacturing practices, pre- and postharvest contamination in seaweed operations can threaten product safety or quality. The aim of the last study was to assess the survival of bacterial pathogens (*Vibrio* spp., *Listeria monocytogenes*, Shigatoxigenic *Escherichia coli* (STEC) and *Salmonella* sp.) in sugar kelp at ambient (22 °C) and refrigerated (4 °C) temperatures, which are typical storage conditions, and a refrigerated temperature-abused (10 °C) sugar kelp.

Results show that inoculated bacteria have limited survival but are capable of surviving on sugar kelp for 7 days, if the starting initial populations are high. These data suggest the need for standardized seaweed growing and processing regulations to minimize the probability of preharvest (*Vibrio* spp. and STEC) and postharvest (*L. monocytogenes* and *Salmonella* sp.) pathogen contaminations. The higher reduction in pathogen population at refrigerated temperature (4 °C) suggests seaweed processors should store or process seaweed at the lowest temperature (4 °C) to minimize pathogen population when seaweeds are contaminated.

Care should be taken during handling and processing of kelp, as the postharvest contaminant *Salmonella* sp. survived best of the species assessed at 4 °C. Although STEC survived to the greatest extent at ambient temperature, the populations of *Vibrio* spp and *Listeria monocytogenes* were higher at the end of ambient storage as compared to refrigerated storage, suggesting that ambient temperature may favor the survival of preharvest pathogens when compared to refrigerated storage. Thus the need to process kelp at lower temperatures. As expected, temperature abuse (10 °C) led to a higher survival of pathogens than refrigerated temperature of 4 °C. These results imply the need for strict adherence to temperature control to ensure kelp safety.

We recommend that similar studies be conducted on other edible seaweed products, and if possible, evaluate additional bacterial food pathogens of public health concern. Also, future study designs should include the monitoring of pH and water activity of the products during storage to confidently predict factors that influence pathogen survival. Results of pathogen survival after storage suggest the need to optimize postharvest processing practices to ensure the safety of sugar kelp by eliminating any

sporadic contaminations that may occur. Further studies on inactivation of these preharvest (e.g. *Vibrio* spp.) and postharvest (e.g. *Salmonella* spp.) pathogens using various preservation methods including thermal inactivation such as blanching will be a significant achievement in ensuring the safety of kelp. Additionally, monitoring procedures at the state level for pathogen detection are highly recommended to better ensure the safety of these products.

In conclusion, to meet the surging demand for edible seaweed, challenges facing the seaweed industry such as short shelf-life and limited products were addressed in this thesis to offer timely information on postharvest practices that will extend the shelf-life of kelp and produce safe, sustainable, high quality, and minimally processed products to support the goals of the developing U.S. industry. Future work on more value-addition of seaweed and microbial challenge studies may help create profitable business opportunities for seaweed producers and processors, and provide safe products to consumers, respectively.

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APPENDICES

APPENDIX A: Additional results and discussion for chapter two (Chapter 2: Effects of pre-freezing blanching procedures on the physicochemical properties and microbial quality of frozen sugar kelp)

Whole blade texture results from month 3 were statistical outliers, thus were not added to the analysis in the main text. Also, data from month 9 were not added to the main text, 1) because most of the results on month 9 were not statistically different from month 12, and 2) to compare results with equal intervals between time points. The effect of pre-freezing blanching procedure on the properties of kelp at months 3 and 9 are shown below.

Textural properties of whole blades: Apart from resilience on month 9, other textural properties were statistically not significant different ($P \leq 0.05$) from each other on month 3 and 9 for blanching temperature (100 °C and 80 °C), time (5 s and 30 s) and method (direct immersion and vacuum packed) as shown in Table A.1.

Table A.1: Texture and drip loss in whole blade sugar kelp at months 3 and 9 of frozen storage [mean \pm SD (n = 3)]

Storage Time	Texture parameters		Hardness (N)			Chewiness			Resilience			% Drip loss		
	Blanching procedures													
M3	Raw		230.7	±	42.5	164.0	±	31.1	0.08	±	0.05	4.0	±	2.4
	Unblanched		107.8	±	22.4 ^a	47.6	±	23.0 ^a	0.004	±	0.004 ^a	1.7	±	0.7 ^a
	DI	80 °C 5s	99.2	±	14.7 ^a	44.4	±	23.5 ^a	0.004	±	0.004 ^a	2.1	±	0.0 ^a
		80 °C 30s	98.3	±	3.8 ^a	58.2	±	11.7 ^a	0.004	±	0.005 ^a	3.3	±	2.7 ^a
		100 °C 5s	67.2	±	29.5 ^a	47.2	±	13.4 ^a	0.002	±	0.002 ^a	15.5	±	6.9 ^a
		100 °C 30s	97.1	±	4.8 ^a	67.6	±	2.1 ^a	0.002	±	0.002 ^a	2.0	±	0.5 ^a
	VP	80 °C 30s	78.6	±	51.1 ^a	61.6	±	36.2 ^a	0.007	±	0.006 ^a	3.0	±	0.6 ^a
		100 °C 30s	71.9	±	22.2 ^a	46.5	±	25.1 ^a	0.004	±	0.005 ^a	2.1	±	0.3 ^a
M9	Unblanched		148.3	±	3.6 ^a	87.5	±	18.1 ^a	0.03	±	0.03 ^a	10.3	±	3.5 ^a
	DI	80 °C 5s	129.2	±	22.0 ^a	90.1	±	29.0 ^a	0.07	±	0.01 ^{ab}	13.8	±	2.9 ^a
		80 °C 30s	172.1	±	48.0 ^a	122.1	±	43.8 ^a	0.04	±	0.03 ^{ab}	10.0	±	0.8 ^a
		100 °C 5s	158.5	±	27.4 ^a	114.8	±	21.4 ^a	0.03	±	0.01 ^{ab}	15.5	±	6.9 ^a
		100 °C 30s	163.0	±	19.9 ^a	114.2	±	16.1 ^a	0.03	±	0.02 ^b	12.2	±	6.5 ^a
	VP	80 °C 30s	151.7	±	27.4 ^a	106.4	±	27.7 ^a	0.06	±	0.01 ^{ab}	20.2	±	6.9 ^a
		100 °C 30s	104.3	±	13.2 ^a	69.0	±	13.5 ^a	0.03	±	0.01 ^{ab}	14.5	±	6.2 ^a

M3 = Month 3, M9 = Month 9, DI = Direct immersion, VP = Vacuum packaged, s = seconds.

Superscripts: different small letters indicate significant difference among treatments within a test period (one-way ANOVA).

Textural properties of shredded slaw: At month 3, a multiway analysis showed that higher blanching temperature (100 °C) and longer blanching time (30 s) decreased the hardness of shredded kelp significantly. However, blanching temperature, time and method had no effect on hardness in shredded samples at month 9 (Table A.2). No significant differences ($P \leq 0.05$) were observed in percent drip loss at both months 3 and 9.

Table A.2: Texture and drip loss in shredded slaw sugar kelp at months 3 and 9 of frozen storage [mean \pm SD (n = 3)]

Storage Time	Texture parameters		Shear force (N) ‘hardness’			% Drip loss		
	Blanching procedures							
M3	Raw		230.7	±	42.5	4.0	±	2.4
	Unblanched		16.8	±	6.5 ^a	9.3	±	3.0
	DI	80 °C 5s	3.5	±	2.6 ^a	9.2	±	3.7
		80 °C 30s	13.0	±	8.5 ^a	6.3	±	3.8
		100 °C 5s	7.5	±	2.0 ^a	7.0	±	3.3
		100 °C 30s	7.2	±	3.4 ^a	9.1	±	1.9
	VP	80 °C 30s	38.2	±	14.8 ^b	9.0	±	6.2
		100 °C 30s	13.4	±	0.9 ^a	10.7	±	2.8
M9	Unblanched		52.2	±	34.1	19.5	±	7.9
	DI	80 °C 5s	23.2	±	5.8	6.8	±	2.4
		80 °C 30s	23.6	±	10.7	10.3	±	4.4
		100 °C 5s	27.1	±	22.2	5.4	±	0.2
		100 °C 30s	25.0	±	3.4	10.1	±	7.9
	VP	80 °C 30s	37.2	±	12.6	14.9	±	5.4
		100 °C 30s	48.3	±	28.8	11.0	±	7.2

M3 = Month 3, M9 = Month 9, DI = Direct immersion, VP = Vacuum packaged, s = seconds.

Superscripts: different small letters indicate significant difference among treatments within a test period (one-way ANOVA).

Color properties of kelp: Product form had no statistically significant effect on color, therefore data for whole blades and shredded slaw were pooled and analyzed together, with mean values reported below. Direct immersion blanching and a higher blanching temperature (100 °C) significantly increased L* and b* values, and decreased a* values as compared to vacuum packaged blanching and lower blanching temperature (80 °C) for both month 3 and 9 samples. At month 9 blanching procedures significantly increased L* values and decreased a* values (Table A.3), which indicate an increase in lightness and greenness of kelp. Most of the samples had a distinct change in color as denoted by ΔE value of 3.0 or above (Silva and Silva, 1999).

Table A.3: Color (Hunter L*, a*, b*) of sugar kelp (both product forms) at months 3 and 9 of frozen storage [mean \pm SD (n = 3)]

Blanching Procedures			L*			a*			b*			ΔE		
M3	Raw		17.5	\pm	1.3	3.3	\pm	1.6	11.8	\pm	1.7	--	\pm	--
	Unblanched		17.7	\pm	1.6 ^{bcd}	2.5	\pm	0.5 ^{ab}	8.8	\pm	2.6 ^a	2.9	\pm	0.6 ^b
		80 °C 5s	17.1	\pm	1.1 ^{cd}	1.4	\pm	1.3 ^{ab}	7.9	\pm	6.6 ^a	5.9	\pm	4.0 ^{ab}
	DI	80 °C 30s	15.2	\pm	1.7 ^d	0.9	\pm	2.2 ^{abc}	11.9	\pm	4.2 ^a	4.3	\pm	2.1 ^{ab}
		100 °C 5s	20.7	\pm	6.3 ^{abc}	3.1	\pm	1.4 ^a	7.6	\pm	10.0 ^a	10.6	\pm	2.1 ^a
		100 °C 30s	23.0	\pm	2.0 ^a	0.9	\pm	1.7 ^{abc}	14.3	\pm	7.2 ^a	8.1	\pm	4.7 ^{ab}
	VP	80 °C 30s	22.6	\pm	1.7 ^{ab}	-0.1	\pm	1.5 ^{bc}	14.0	\pm	3.1 ^a	7.4	\pm	1.0 ^{ab}
M9		100 °C 30s	22.9	\pm	2.5 ^{ab}	-1.4	\pm	1.2 ^c	11.1	\pm	3.0 ^a	7.7	\pm	2.0 ^{ab}
	Unblanched		20.3	\pm	2.2 ^c	2.9	\pm	0.4 ^a	14.4	\pm	3.3 ^d	4.1	\pm	3.4 ^d
		80 °C 5s	23.2	\pm	1.9 ^{abc}	-1.0	\pm	1.1 ^{bc}	19.8	\pm	2.4 ^{bc}	11.0	\pm	1.3 ^{abc}
	DI	80 °C 30s	25.2	\pm	1.4 ^a	-0.4	\pm	1.5 ^{bc}	25.1	\pm	2.0 ^a	16.0	\pm	2.3 ^a
		100 °C 5s	24.9	\pm	3.0 ^a	-1.0	\pm	1.1 ^{bc}	24.6	\pm	2.7 ^a	15.7	\pm	3.6 ^{cd}
		100 °C 30s	24.4	\pm	1.3 ^{ab}	-1.2	\pm	1.5 ^c	24.3	\pm	2.8 ^{ab}	15.7	\pm	2.2 ^{ab}
	VP	80 °C 30s	22.1	\pm	0.9 ^{abc}	0.9	\pm	0.9 ^{ab}	17.0	\pm	1.8 ^{cd}	7.6	\pm	1.9 ^{ab}
		100 °C 30s	21.4	\pm	1.0 ^{bc}	-0.3	\pm	1.2 ^{bc}	18.9	\pm	2.5 ^c	9.1	\pm	0.8 ^{bcd}

Table indicates pooled average of shredded slaw and whole blade kelp

Hunter (L*, a*, b*): L* = lightness, a*= red/green, b*=yellow/blue, ΔE =Change in color

M3 = Month 3, M9 = Month 9, DI = Direct immersion, VP = Vacuum packaged, s = seconds.

Superscripts: different small letters indicate significant difference among treatments within a test period (one-way ANOVA).

Moisture and microbial qualities of kelp: Moisture in kelp was not analyzed at months 3 and 9. All blanched and shredded slaw had higher percent moisture as compared to unblanched (Table A.4). Higher blanching temperature (100 °C) resulted in a higher percent moisture as compared to lower blanching temperature for all treatments in both product forms. Blanching method did not have significant impact on moisture content in both product form contrary to results observed in the other three time points (day 1, month 6 and 12), where direct immersion significantly increased moisture content as compared to vacuum packed samples.

Blanching temperature and time, as well as blanching method, had no significant effect on APC, psychrotroph and fungi of samples at months 3, 6 and 9. This follows the trend observed in samples from the other time points as stated in the text. All psychrotroph and fungi counts were below 2.2 log CFU/g and APC counts were all below 3.2 log CFU/g. The low microbial counts observed at months 3, 6 and 9 suggest a minimal risk of bacterial or fungal spoilage during frozen storage.

Table A.4: Moisture content and microbial counts¹ of sugar kelp at months 3 and 9 of frozen storage [mean \pm SD (n = 3)]

Storage Time	Blanching procedures		Moisture (%, ww) for whole blades	Moisture (%, ww) for shredded slaw	APC (Log CFU/g) ¹	Psychrotroph (Log CFU/g) ¹	Fungi (Log CFU/g)
M3	Raw		88.6 ± 0.9	88.5 ± 0.4	2.00 ± 0.00 ^a	2.00 ± 0.00 ^a	2.00 ± 0.00 ^a
	Unblanched				2.00 ± 0.00 ^a	2.00 ± 0.00 ^a	2.00 ± 0.00 ^a
	DI	80 °C 5s			2.15 ± 0.16 ^a	2.00 ± 0.00 ^a	2.00 ± 0.00 ^a
		80 °C 30s			2.00 ± 0.00 ^a	2.00 ± 0.00 ^a	2.00 ± 0.00 ^a
		100 °C 5s	N/A	N/A	2.05 ± 0.12 ^a	2.00 ± 0.00 ^a	2.00 ± 0.00 ^a
		100 °C 30s			2.05 ± 0.12 ^a	2.00 ± 0.00 ^a	2.00 ± 0.00 ^a
	VP	80 °C 30s			2.10 ± 0.15 ^a	2.00 ± 0.00 ^a	2.00 ± 0.00 ^a
		100 °C 30s			2.05 ± 0.12 ^a	2.00 ± 0.00 ^a	2.00 ± 0.00 ^a
M6	Unblanched		88.4 ± 1.4 ^{bc}	88.1 ± 2.4 ^c	2.82 ± 0.66 ^a	2.00 ± 0.00 ^a	2.04 ± 0.04 ^a
	DI	80 °C 5s	92.7 ± 1.0 ^a	90.9 ± 0.4 ^{abc}	2.79 ± 0.52 ^a	2.00 ± 0.00 ^a	2.00 ± 0.00 ^a
		80 °C 30s	92.0 ± 1.9 ^{abc}	91.0 ± 1.3 ^{abc}	3.18 ± 0.72 ^a	2.00 ± 0.00 ^a	2.00 ± 0.00 ^a
		100 °C 5s	92.4 ± 1.1 ^{ab}	93.1 ± 1.6 ^{ab}	2.85 ± 0.88 ^a	2.00 ± 0.00 ^a	2.00 ± 0.00 ^a
		100 °C 30s	93.5 ± 0.5 ^a	93.6 ± 0.9 ^a	3.04 ± 0.47 ^a	2.10 ± 0.16 ^a	2.00 ± 0.00 ^a
	VP	80 °C 30s	88.0 ± 2.6 ^c	88.4 ± 0.9 ^{bc}	2.81 ± 0.57 ^a	2.00 ± 0.00 ^a	2.00 ± 0.00 ^a
		100 °C 30s	90.8 ± 1.2 ^{abc}	90.0 ± 1.1 ^{abc}	3.06 ± 1.17 ^a	2.00 ± 0.00 ^a	2.00 ± 0.00 ^a
	M9	Unblanched				2.38 ± 0.35 ^a	2.00 ± 0.00 ^a
DI		80 °C 5s			2.28 ± 0.23 ^a	2.00 ± 0.00 ^a	2.00 ± 0.00 ^a
		80 °C 30s			2.18 ± 0.21 ^a	2.00 ± 0.00 ^a	2.00 ± 0.00 ^a
		100 °C 5s	N/A	N/A	2.56 ± 0.25 ^a	2.00 ± 0.00 ^a	2.05 ± 0.12 ^a
		100 °C 30s			2.15 ± 0.25 ^a	2.00 ± 0.00 ^a	2.00 ± 0.00 ^a
VP		80 °C 30s			2.39 ± 0.41 ^a	2.00 ± 0.00 ^a	2.00 ± 0.00 ^a
		100 °C 30s			2.30 ± 0.41 ^a	2.00 ± 0.00 ^a	2.00 ± 0.00 ^a

¹ Microbial counts (mean \pm s.d.) of sugar kelp for both product forms.

M3, month 3; M9, month 9; DI, direct immersion; VP, vacuum packaged; s, seconds

Superscripts: different small letters indicate significant differences among treatments within a test period

Absence of capital letters indicates no significant differences during storage within a specific treatment across 12-month frozen storage (one-way ANOVA).

CFU, coliform forming units; APC, aerobic plate count; Fungi, yeast and molds.

APPENDIX B: Supplementary tables showing the model effect (*P*-values) on the qualities of sugar kelp during 12 months frozen storage

Table B.1: Model effect (*P*-values) on the qualities of whole blade sugar kelp during 12 months frozen storage

Dependent variables	Whole blades					
	Frozen Storage	Frozen Storage	Frozen Storage	Blanching Temp.	Blanching Temp.	Blanching Method
	× Blanching Method	× Blanching Temp.	× Blanching Time	× Blanching Time	× Blanching Method	× Blanching Time
Color						
L*	0.600	0.452	0.092	< 0.000	0.188	-
a*	0.641	0.654	0.656	0.008	0.106	-
b*	0.039	0.044	0.590	< 0.000	< 0.000	-
Texture						
Hardness	0.620	0.483	0.807	0.370	0.367	-
Chewiness	0.629	0.765	0.568	0.317	0.492	-
Resilience	0.850	0.060	0.424	0.386	0.042	-
Chemical & Physical						
Moisture	0.797	0.251	0.991	0.366	0.745	-
% Drip loss	0.581	0.677	0.848	0.298	0.871	-
Ash	0.854	0.995	0.463	0.339	0.777	-
Calcium	0.290	0.472	0.154	0.164	0.437	-
Magnesium	0.933	0.434	0.483	0.513	0.821	-
Potassium	0.932	0.515	0.535	0.743	0.135	-
Sodium	0.512	0.441	0.663	0.974	0.745	-
TPC	N/A	N/A	N/A	0.891	0.204	-
FRAP	N/A	N/A	N/A	0.759	0.554	-
Microbial						
APC	0.967	0.643	0.996	0.580	0.879	-
Psychrotrophs	-	-	-	-	-	-
Fungi	0.792	0.911	0.408	0.384	0.586	-

Bold numbers: Significant, N/A = Not applicable (did not analyze), - = No result (No results generated after statistical analysis).

Table B.2: Model effect (*P*-values) on the qualities of shredded kelp during 12 months frozen storage

Dependent variables	Shredded slaw					
	Frozen Storage	Frozen Storage	Frozen Storage	Blanching Temp.	Blanching Temp.	Blanching Method
	× Blanching Method	× Blanching Temp.	× Blanching Time	× Blanching Time	× Blanching Method	× Blanching Time
Color						
L*	0.282	0.378	0.880	0.159	0.165	-
a*	0.204	0.843	0.924	0.057	0.001	-
b*	0.047	0.218	0.304	0.001	< 0.000	-
Texture						
Hardness	0.822	0.801	0.431	0.379	0.072	-
Chemical & Physical						
Moisture	0.046	0.983	0.294	0.039	0.004	-
% Drip loss	0.944	0.652	1.000	0.680	0.622	-
Ash	0.824	0.951	0.557	0.005	0.001	-
Calcium	0.444	0.588	0.822	0.562	0.426	-
Magnesium	0.667	0.446	1.000	0.074	0.004	-
Potassium	0.355	0.425	0.564	0.010	< 0.000	-
Sodium	0.994	0.490	0.561	0.028	0.002	-
TPC	N/A	N/A	N/A	0.551	0.035	-
FRAP	N/A	N/A	N/A	0.860	0.980	-
Microbial						
APC	0.694	0.301	0.990	0.057	0.252	-
Psychrotrophs	0.006	0.284	0.004	0.015	0.021	-
Fungi	0.513	0.859	0.569	0.388	0.415	-

Bold numbers: Significant, N/A = Not applicable (did not analyze), - = No result (No results generated after statistical analysis).

APPENDIX C: pH during kelp and/or cabbage sauerkraut fermentation

The pH of all sauerkraut treatments was monitored to track the fermentation process. Sauerkraut samples from each fermentation jar ($n = 3$) of the five treatments (raw, raw/frozen, blanched, blanched/frozen and 100% cabbage) were taken during fermentation on days 0, 3, 6 and 9 after the start of fermentation. The decrease in pH of the sauerkraut was measured by a pH meter (Thermo Scientific™ Orion Star™ A111 pH Benchtop Meter, Waltham, MA) with a flat probe attachment (Thermo Scientific™ Orion™ AquaPro™ Flat Surface 9135, Waltham, MA). Approximately 10 g of the kelp sauerkraut were aseptically removed from the fermentation jars. The flat probe was placed directly on the kelp sauerkraut until a consistent reading was obtained. Measurements were taken in duplicate per fermentation jar and pH values were averaged.

The pH value of the cabbage only sauerkraut samples reached a pH of 4 or lower by day six of fermentation and this fermentation time was relatively longer as compared to other cabbage fermentation by *Listeriaplantarum* (Hunaefi et al. 2013). However, kelp/cabbge sauerkraut fermentation reached a a pH of 4 or lower earlier as compared to another kelp cabbage mix fermentation study (Skonberg et al. 2021) and a cabbage sauerkraut fermentation (Beganović et al. 2014) that took approximately 15 days and 28 days, respectively.

Table C.1: Mean pH values of cabbage- and kalp/cabbage sauerkraut over time during fermentation ($n = 3$).

Treatments	Day 0	Day 3	Day 6	Day 9
Cabbage only	5.93 ± 0.04	4.53 ± 0.02	3.91 ± 0.02	--
Raw kelp	5.75 ± 0.02	5.13 ± 0.01	4.31 ± 0.08	3.51 ± 0.14
Raw/frozen kelp	5.76 ± 0.04	5.04 ± 0.03	4.09 ± 0.03	3.63 ± 0.03
Blanched kelp	5.74 ± 0.02	4.89 ± 0.02	4.14 ± 0.04	3.80 ± 0.01
Blanched/frozen kelp	5.71 ± 0.03	4.79 ± 0.03	4.31 ± 0.04	3.84 ± 0.03

APPENDIX D: Informed consent for sensory evaluation

1. Informed consent for consumer acceptability of sugar kelp (seaweed) salad

You are invited to take part in a research project titled “**Sustainable Post-harvest Processing and Value-addition of Aquaculture Seaweed**” by Samuel Akomea-Frempong, who is a doctoral student in the School of Food and Agriculture at the University of Maine. He is advised by faculty members Jennifer Perry and Mary Ellen Camire. The purpose of the research is to learn if consumers prefer blanched seaweed to raw seaweed. Blanching is a brief exposure of vegetables to hot water. You must be at least 18 years old to take part in this project. If you are allergic to seaweed, carrots, or sesame seeds, or any of the ingredients of Asian vinaigrette including (balsamic vinegar, vegetable oil (soybean and/or canola), extra virgin olive oil, salt, garlic, spice, onion, xanthan gum, red bell pepper, mustard flour), or do not enjoy eating seaweed, please do not take part in this study.

What Will You Be Asked to Do?

If you choose to take part in this study, you will be asked to answer a few questions about yourself. Then, you will be served three samples of refrigerated seaweed salad. For each sample, you will be asked to rate how much you like that sample. You will be asked to take several bites to evaluate the samples. The test may take about 15 minutes to complete.

Risks

Except for your time and inconvenience, there are no risks to you from taking part.

Benefits

You may enjoy eating the seaweed salad. While there are no direct benefits to you, this research may help Maine seaweed growers and processors develop new products.

Compensation

Upon completion of today’s test, you will receive \$5. No compensation will be provided if you decide not to complete the test.

Confidentiality

Your answers will be collected anonymously. Your name will not be on any files that contain your answers to our questions. Data will be kept indefinitely in the University’s Digital Commons site.

Voluntary

Taking part in this study is voluntary. If you choose to take part in this study, you may stop at any time or skip questions, but you will not receive any compensation.

Contact Information

If you have any questions about this study, please contact me at Samuel.akomeafrempong@maine.edu or (207) 889-1970, or Professor Camire at camire@maine.edu or (207) 581-1733. If you have any questions about your rights as a research participant, please contact the Office of Research Compliance, University of Maine, at 207/581-2657 (or e-mail umric@maine.edu).

2. Informed consent for consumer acceptability of sugar kelp (seaweed) sauerkraut

You are invited to take part in a research project titled “**Sustainable Post-harvest Processing and Value-addition of Aquaculture Seaweed**” by Samuel Akomea-Frempong, who is a doctoral student in the School of Food and Agriculture at the University of Maine. He is advised by faculty members Jennifer Perry and Mary Ellen Camire in the School of Food and Agriculture. You must be at least 18 years old to take part in this project. If you do not like sauerkraut, seaweed, cabbage, or fermented vegetables, or are allergic to seaweed or cabbage, please do not take part in this study.

What Will You Be Asked to Do?

If you choose to take part in this study, you will be asked to answer a few questions about yourself. Then, you will be served three samples of refrigerated sauerkraut, with and without seaweed. For each sample, you will be asked to rate how much you like that sample. You will be asked to take several bites to evaluate the samples. The test may take about 15 minutes to complete.

Risks

Except for your time and inconvenience, there are no risks to you from taking part.

Benefits

You may enjoy eating the seaweed sauerkraut. While there are no direct benefits to you, this research may help Maine seaweed growers and processors develop new products.

Compensation

Upon completion of today’s test, you will receive \$5. No compensation will be provided if you decide not to complete the test.

Confidentiality

Your answers will be collected anonymously. Your name will not be on any files that contain your answers to our questions. Data will be kept indefinitely in the Sensory Evaluation Center’s locked office.

Voluntary

Taking part in this study is voluntary. If you choose to take part in this study, you may stop at any time or skip questions, but you will not receive any compensation. Some questions like have a “prefer not to answer” option. Please answer all of the questions that have to do with evaluating the sauerkraut.

Contact Information

If you have any questions about this study, please contact me at samuel.akomeafrempong@ maine.edu or (207) 889-1970, or Professor Camire at camire@maine.edu or (207) 581-1733. If you have any questions about your rights as a research participant, please contact the Office of Research Compliance, University of Maine, at 207/581- 2657 (or e-mail umric@maine.edu).

APPENDIX E: Consumer acceptability of sugar kelp (seaweed) salad recruitment notice

Are you interested in trying sugar kelp salad?

If you are at least 18 years old and like eating seaweed, please help University of Maine researchers evaluate minimally processed (blanched) and raw seaweed salads.

Testing will take about 15 minutes, and you will be paid \$5 for completing the survey of how much you like three seaweed samples. You will be asked to take several bites of the samples.

If you do not like seaweed, or have allergies to seaweed, carrots, sesame seed and Asian vinaigrette salad dressing including balsamic vinegar, vegetable oil (soybean and/or canola), extra virgin olive oil, salt, garlic, spice, onion, xanthan gum, red bell pepper and mustard flour please do not participate.

Testing will be held on: April 24th 2019 from 11:00 am to 5:00 pm

Please sign up for the test using this link: (Doodle poll link inserted here). Alternatively, you can email the principal researcher Samuel Akomea-Frempong, a PhD student in Food and Nutritional Sciences at samuel.akomeafrempong@maine.edu to schedule an appointment for this study, or for more information.

Location: 158 Hitchner Hall (Sensory Evaluation Center)

APPENDIX F: Consumer acceptability of seaweed sauerkraut recruitment notice

Are you interested in trying sauerkraut containing a locally-grown seaweed?

You are being contacted because you chose to be notified about testing being conducted by the University of Maine Sensory Evaluation Center.] If you are at least 18 years old and like eating seaweed, please help University of Maine researchers evaluate a research study on minimally-processed seaweed sauerkraut.

Testing will take about 15 minutes, and you will be paid \$5 for completing the survey of how much you like three seaweed samples. You will be asked to take several bites of the samples. If you do not like sauerkraut, fermented vegetables, or seaweed, or have allergies to seaweed or cabbage, please do not participate.

Testing will be held on: TBD

Please sign up for the test using this link: (Doodle poll link inserted here). Alternatively, you can email the principal researcher Samuel Akomea-Frempong, a PhD student in the School of Food and Agriculture at samuel.akomeafrempong@maine.edu to schedule an appointment for this study, or for more information.

Location: 158 Hitchner Hall (Sensory Evaluation Center)

APPENDIX G: Consumer acceptability questionnaires for kelp salad and sauerkraut

1. Consumer acceptability questionnaires for sugar kelp (seaweed) salad

Thank you for participating. Please answer some questions about yourself, then evaluate all three samples, in order from left to right. Take a sip of water before tasting each sample. Make sure that the sample code on the sample you are trying matches the code on the computer screen.

Please indicate your gender.

- ☐ Male
- ☐ Female
- ☐ Prefer to not answer

Please indicate your age bracket based on your last birthday.

- ☐ 18- 25
- ☐ 26- 35
- ☐ 36-45
- ☐ 46-55
- ☐ 56 years or older
- ☐ Prefer not to answer

Please indicate the racial group you identify with.

- ☐ American Indian/Alaska Native
- ☐ Asian
- ☐ Black/African American
- ☐ White (Caucasian)
- ☐ Native Hawaiian/Other Pacific Islander
- ☐ Prefer not to answer

Where do you usually consume seaweed?

- ☐ At a restaurant
- ☐ At home
- ☐ Other
- ☐ Not applicable

Approximately how often do you consume seaweed?

- ☐ 1-2 times a week
- ☐ 1-2 times a month
- ☐ Every 2-3 months
- ☐ 1-2 times a year
- ☐ Weekly
- ☐ 2 or more times a week

Would you like to consume your seaweed raw?

- ☐ Yes
- ☐ No

What form of seaweed products do you typically consume?

- ☐ Salad
- ☐ Frozen smoothie cubes
- ☐ Soup
- ☐ As part of other foods like sushi
- ☐ Other.....

What would make you consume seaweed more often? (Select all that apply)

- ☐ Lower price
- ☐ More availability
- ☐ Longer shelf life
- ☐ Sustainably-grown
- ☐ Minimally processed
- ☐ Sold fresh
- ☐ Sold in ready-to-eat dishes

How much would you pay for a ready-to-eat four-ounce (4 oz) seaweed salad bowl?

- ☐ Would not buy
- ☐ USD 2.00
- ☐ USD 3.00
- ☐ USD 4.00
- ☐ USD 5.00

Which sensory characteristic of seaweed is most important to you? Please choose only one answer.

- ☐ Flavor
- ☐ Texture
- ☐ Color
- ☐ Aroma
- ☐ Other: _____

Please evaluate the first sample.

[Note: These questions will be repeated for each sample.]

How much do you like the appearance of this sample?

- ☐ Dislike Extremely
- ☐ Dislike Very Much
- ☐ Dislike Moderately
- ☐ Dislike Slightly
- ☐ Neither Like nor Dislike
- ☐ Like Slightly
- ☐ Like Moderately
- ☐ Like Very Much
- ☐ Like Extremely

How much do you like the color of this sample?

- ☐ Dislike Extremely
- ☐ Dislike Very Much
- ☐ Dislike Moderately
- ☐ Dislike Slightly
- ☐ Neither Like nor Dislike
- ☐ Like Slightly
- ☐ Like Moderately
- ☐ Like Very Much
- ☐ Like Extremely

Please take a bite and evaluate the texture questions below.

How much do you like the texture of this sample?

- ☐ Dislike Extremely
- ☐ Dislike Very Much
- ☐ Dislike Moderately
- ☐ Dislike Slightly
- ☐ Neither Like nor Dislike
- ☐ Like Slightly
- ☐ Like Moderately
- ☐ Like Very Much
- ☐ Like Extremely

How would you rate the firmness of this sample?

- ☐ Not firm
- ☐ Somewhat firm
- ☐ Just about right
- ☐ Somewhat too firm
- ☐ Much too firm

How would you rate the tenderness of this sample?

- ☐ Not chewy
- ☐ Somewhat chewy
- ☐ Just about right
- ☐ Somewhat too chewy
- ☐ Much too chewy

Which one word best describes the texture of this sample? (choose one)

- ☐ Tender
- ☐ Chewy
- ☐ Tough
- ☐ Mushy
- ☐ Soft
- ☐ Firm
- ☐ Juicy
- ☐ Dry

Please take another bite and evaluate the flavor and overall liking.

How much do you like the flavor of this sample?

- ☐ Dislike Extremely
- ☐ Dislike Very Much
- ☐ Dislike Moderately
- ☐ Dislike Slightly
- ☐ Neither Like nor Dislike
- ☐ Like Slightly
- ☐ Like Moderately
- ☐ Like Very Much
- ☐ Like Extremely

How much do you like the sample overall?

- ☐ Dislike Extremely
- ☐ Dislike Very Much
- ☐ Dislike Moderately
- ☐ Dislike Slightly
- ☐ Neither Like nor Dislike
- ☐ Like Slightly
- ☐ Like Moderately
- ☐ Like Very Much
- ☐ Like Extremely

Is there anything else that you would like to say about this sample? Please type the sample's three-digit code in your comments.

Comment Box

Thank you for your time and opinions. Please raise the window slightly to let the kitchen staff know that you are done.

2. Consumer acceptability questionnaires for sugar kelp (seaweed) sauerkraut

Thank you for participating. Please answer some questions about yourself, then evaluate all three samples, in order from left to right. Take a sip of water before tasting each sample. Make sure that the sample code on the sample you are trying matches the code at the top of the computer screen.

Please indicate your gender.

- ☐ Male
- ☐ Female
- ☐ Others
- ☐ Do not want to answer

Please indicate your age bracket based on your last birthday.

- ☐ 18- 25
- ☐ 26- 35
- ☐ 36-45
- ☐ 46-55
- ☐ 56-65
- ☐ 66 years or older
- ☐ Prefer not to answer

Please indicate the racial group you identify with.

- ☐ American Indian/Alaska Native
- ☐ Asian
- ☐ Black/African American
- ☐ White (Caucasian)
- ☐ Native Hawaiian/Other Pacific Islander
- ☐ Prefer not to answer

About how often do you consume sauerkraut?

- ☐ Less than once per year
- ☐ 1-6 times per year
- ☐ 1-2 times per month
- ☐ 1 or more times per week

About how often do you consume seaweed?

- ☐ Less than once per year
- ☐ 1-6 times per year
- ☐ 1-2 times per month
- ☐ 1 or more times per week

Did you know that fermented foods, such as sauerkraut, contain probiotics that are associated with disease prevention and improved digestion?

- ☐ Yes
- ☐ No

About how often do you eat foods or dietary supplements containing probiotics?

- ☐ Less than once per year
- ☐ 1-4 times per year
- ☐ 1-2 times per month
- ☐ 1-2 times per week
- ☐ 3+ times per week

Please evaluate the samples in the order indicated on your screen and verify that the three-digit code matches the current sample being tested as you rate each sample. Please take a sip of water before tasting each sample.

How much do you like the appearance of this sample?

- ☐ Dislike extremely
- ☐ Dislike very much
- ☐ Dislike moderately
- ☐ Dislike slightly
- ☐ Neither like nor dislike
- ☐ Like slightly
- ☐ Like moderately
- ☐ Like very much
- ☐ Like extremely

How much do you like the color of this sample?

- ☐ Dislike extremely
- ☐ Dislike very much
- ☐ Dislike moderately
- ☐ Dislike slightly
- ☐ Neither like nor dislike
- ☐ Like slightly
- ☐ Like moderately
- ☐ Like very much
- ☐ Like extremely

How much do you like the aroma (smell) of this sample?

- ☐ Dislike extremely
- ☐ Dislike very much
- ☐ Dislike moderately
- ☐ Dislike slightly
- ☐ Neither like nor dislike
- ☐ Like slightly
- ☐ Like moderately
- ☐ Like very much
- ☐ Like extremely

How much do you like the flavor of this sample?

- ☐ Dislike extremely
- ☐ Dislike very much
- ☐ Dislike moderately
- ☐ Dislike slightly
- ☐ Neither like nor dislike
- ☐ Like slightly
- ☐ Like moderately
- ☐ Like very much
- ☐ Like extremely

How much do you like the texture of this sample?

- ☐ Dislike extremely
- ☐ Dislike very much
- ☐ Dislike moderately
- ☐ Dislike slightly
- ☐ Neither like nor dislike
- ☐ Like slightly
- ☐ Like moderately
- ☐ Like very much
- ☐ Like extremely

How much do you like this sample overall?

- ☐ Dislike extremely
- ☐ Dislike very much
- ☐ Dislike moderately
- ☐ Dislike slightly
- ☐ Neither like nor dislike
- ☐ Like slightly
- ☐ Like moderately
- ☐ Like very much
- ☐ Like extremely

Please check any word that you think describes this sample. You may check all that apply. [Note: terms will be randomized in order.]

fresh	crunchy	mushy	soggy
salty	sweet	sour	bitter
crunchy	fishy	bland	metallic
traditional kraut	pickled	slimy	mild
ocean breeze	soft	boiled cabbage	brackish
well-rounded	musty	clean	fizzy
tangy	nutty	pungent	mellow

Is there anything else you would like to tell us about this sample? If you refer to other samples in this test, please use those samples' three-digit codes.

Thank you very much for your time and opinions. Please raise the window slightly to let the staff know that you are done, and do not forget to pick up your incentive.

APPENDIX H: Panelists' comments on samples during sensory evaluation

Summary comments report for of sugar kelp (seaweed) salad

Sample coded 479: Raw kelp salad (control).

Sample coded 673: Blanched kelp for 1 min.

Sample coded 275: Blanched kelp for 3 min.

Sample	Comment
Raw	something off in 479 compared to 275. Some bitter off taste
	There was an odd musty smoky flavor that was not as nice as the other two samples
	It's a little hard determining the firmness of the seaweed with the firm carrots mixed in
	Sample 479 is too chewy and bitter. The bitterness gets worse as you eat more of it
	I liked that the seaweed was firm, not mushy or slimy. Not too salty.
	tasted grassy but in a good way
	The best one in my opinion was 275. 479 was definitely better than 673 but still had bit of an after taste I was not suspecting
	Too mild for my liking, and a little too soft--I would rather have more crunch
	This sample had a slightly more bitter flavor compared to 275 and 673
	somehow better than 673, still want more vinegar, ditto on lengths
	The color is not so appearing. The texture turns out great, but the flavor is not too good.
	I like everything but the flavor, which is why I rated it overall as a dislike. It looks great and has a nice al dente texture, but leaves a bad, possibly bitter, taste in my mouth
	this sample tastes like my lawnmower smells
	Just from the appearance of sample 479, I took a smaller taste than I did for the other two samples that didn't look bad for appearance rating
	is sour and not chewy enough
	Sample 479 had a nice flavor, but I would like it a little more salty. The color is similar to any other seaweed and the texture was soft, smooth which is what I prefer.
	Sample 479 was very good. Loved the flavor.

	The flavor of sample 479 was more bitter than 275 and 673. The color was darker and less appealing than the brighter greens of 673.
	My favorite of the 3, the other two had stronger taste
	The texture is great. I wished it was brighter green. And it would be tastier if the salad dressing was a little sweeter
	It tasted the most like the ocean. Needs more flavor and is too chewy. There was a slight bitterness.
	the addition of the carrot makes it appealing, and the salad dressing makes it yummy
	This sample tastes a bit bitter
	I wish it was just a lit bit more flavorful
	lack of flavor in this one
	The pieces are too darn long I had to slurp them like spaghetti!
	479 is bitter as compared to 275
	The texture and color are good but there is an off-slightly bitter flavor to the salad
	All 3 samples were good, but I think I would eat seaweed salad with a dressing of some sort, like maybe a sweet sauce to counteract the salty nature of the seaweed
	This sample has strong flavor which is good to me!
	This salad has a lightly lemony aftertaste. I like it. However, it still tastes pretty similar to 673 and 275.
	Sample 479 takes a little too long to chew
	has a more noticeable (bad) aftertaste which kind of killed the experience for me. Visually, it doesn't look as appealing either.
	addition and thought that this sample was slightly less chewy than the previous (275) sample
	479 would be my second favorite of the three, I didn't like the chewiness of 275 but the firmness of 479 was a tad to firm.
	479 had too chewy of a texture and a strong off-flavor, not sure if it was the dressing or the sea vegetables.
	Very unique - but very pleasant! Bit of a burn on the back of the throat after a minute or so...
	had the texture and crunch, but the after taste is bitter, almost like when a vegetable hasn't been washed after you buy it
	Sample #479: This one is more spicy than the last two (275 & 673), I feel. Strong carrotty flavor, & something else savory I can't quite identify. I like it a lot, but feel like the extra ingredients might be obscuring the seaweed
	less than with 673. They are all delicious to eat. I think that overall, this might tie with 673 for my favorite? I have no

	problem with any of them though. Honestly which I like best might have more to do with which one I had most
	recently than with anything else. All are delightful. I prefer them over the one sold in the memorial union, which I found a bit sweeter than I prefer, I think. (Not that it is bad either, just different degrees of delicious.)
	Prefer 275 over 479 taste wise. Seems like it's missing a component like sweetness or just a different flavor.
	Sample 479 had a pleasing texture. It had the firmness and chew that I like about seaweed salads. However, the flavor was overpowered by the carrots. Overall, I felt like it could use more dressing and a brighter (more acidic) flavor
	to balance out the earthiness of the carrots and the seaweed
	479 Better, needs salt, this sample has more carrots (I like carrots, better than the seaweed). I might like seaweed in general if there were less of it in a salad. It does add an additional layer of favor.
	479 still has a bitter aspect but not as aggressively as 673. Also, with all the strandlets getting caught in my teeth, I'm not sure how much the water is doing to truly cleanse my palate.
	Sample 479 was my least favorite in terms of taste and texture. It had a more bitter flavor (a bit more overpowering of a seaweed taste) and the texture was drier than the other two (275 and 673). I thought the color of this one was
	the best of the three, however
	I think I generally like seaweed salad more tangy, but this was ok (not too salty, not slimy, etc). Like the firm crunch of the seaweed and the carrot
	Sample 479 tastes more bitter than sample 673 did; it starts out similarly yummy, and the bitterness comes on midway through and finishes that way. Maybe the pieces were cut a little larger for 479 than for 673, but 479 felt a little slimier in my mouth.
	479 - the clumpiness of the salad made it hard to get a bite size portion, and the length of the seaweed was also too long for getting a reasonable portion on your fork. There was a slight bitterness at first, but then a good taste probably due to the dressing
Blanched for 1 min	The initial flavor of the sample was a bit overpowering, the underlying seaweed and sesame flavor was nice
	673 strands were long and difficult to take bites (similar to 275)
	673 was way too stringy. You had to roll it up on your fork like it was pasta. I didn't like that consistency of it

	Had a bit of a blast of flavor in the end I was definitely not expecting. Was not a pleasant surprise.
	really enjoyed this one. great flavor and a little crunch while maintaining tenderness
	I'd add more sugar, vinegar to dressing and also make sure pieces were mostly either all long like pasta, or more uniformly short, rather than straggler long pieces
	a little bland
	doesn't leave a bitter taste in my mouth like sample 479. nice and smooth.
	Sample 673 tastes slightly less like yard trimmings than sample 479 did.
	Sample 673 has a less pleasing taste than the other two samples.
	673 - there is a taste that stands out but I'm not sure what leaves a weird after taste in your mouth
	673 is sour and not chewy enough
	The flavor left a slight bitter aftertaste. The texture was soft, the color similar to any other seaweed. I would prefer if it was a little bit less chewy.
	Sample 673 has a nice crunch that was lacking from 275.
	Something about the texture is offputting
	The texture for all three sample were similar as they were crunchy, i like my seaweed crunchy. The color was more pleasing than sample 479.
	673: Flavor is much better than 275. Nice sesame crunch. Could use some more acidity.
	Appearance a little off-putting Sample 275 had best flavor profile Aftertaste not overpowering/mild
	maybe a tad more sesame oil.
	This one has a nasty aftertaste
	Prominent ginger flavor which I enjoy
	This seaweed salad is very aromatic and flavorful. I would buy this salad.
	The flavor or sample 673 is less appetizing. It is too acidic.
	Sample 673 tastes better eaten in small volumes; I've never had seaweed essentially by itself before but I thoroughly enjoyed the texture of this first sample.
	This was my favorite sample from this session. While I still feel that the seaweed pieces were longer than I would have liked, I found the firmness to be more desirable than the previous two samples. I also thought the spice and flavor profile of this samples was more pleasant than the previous samples.

	673 didn't have as much flavor as I liked in 275, but the texture was slightly improved for my tastes.
	had a strong `sea` flavor and the texture was too chewy.
	very firm, but texture still very acceptable
	a bit of a bad after taste
	673 is the best one
	This one seems more strongly flavored with a bit more gingery, bitter flavor. It is also very delicious! I think I might prefer this one since it has a stronger flavor, but they are both very good. (I'm partial to foods with strong flavor.)
	Too salty
	This sample was just right in firmness and texture - had good flavor and did not smell as sea weedy as #479.
	Sample 673 doesn't quite have that good chew that I like about seaweed salads. The flavor was good- I was able to taste more of the dressing in this sample than samples 275 and 469. However, I think the dressing could use more brightness and sesame flavor. The carrots distract from the seaweed and dressing.
	673 Softer, needs salt, not quite as sweet.
	673 has a bitter taste. I had a very long string of seaweed at one point and because of the texture had a moment where I wondered if I'd need to use my hand to help break the strand.
	I thought this sample (673) was very similar in all respects to 275. The texture was maybe slightly less crunchy and juicy but still very good. I thought the color was also about the same.
	Found this one (673) to be chewy / a bit slimy. Liked that I tasted some ginger but otherwise 275 is still my favorite for tangy flavor and nice texture. I find 479 pretty bland in comparison, but texture good
	At first I thought firmness and tenderness would be on the same spectrum. I know sample 673 took a while to chew, but I'm not sure if that meant it was firm or not. Since they were separate questions, I interpreted firm to mean soft or firm, and tenderness to relate to the chewy or mushy. That's how I answered the questions. I liked the flavor a lot!
	673 - of the three samples (479, 257) this one seemed to have the more dressing, which was almost too much. No bitterness with this sample. No real issues with clumpiness, which may be due to the amount of dressing, not sure.
Blanched for 3 min	There were more carrots in this sample which was very nice
	I like sample 275 the best because it didn't have as much bitter flavor as the other two samples and I liked how it had a little

	crunch to it. Unlike the other two samples though, sample 275 gets stuck in your teeth more.
	275 had a good mouth feel--enough snap but not too chewy, decent flavor.
	275 it stayed balled up so it was hard to take individual bites. Some of them were REALLY long which made it messy to eat.
	275 was too clumped together to be as appealing as I wanted it to be. It made it less appealing before I even began eating it.
	sample 275: nice variations of color and a nice flavor. would prefer more crunch--i think one could achieve a better balance between tenderness and crunch
	The sample was pretty firm (crunchy ?), however for my personal preference I feel that I would not have enjoyed it as much if it was less firm.
	seemed a bit better in flavor than the others; also fewer long strings hanging out of my mouth
	I found this just tough and hard for me chew up. The firmness of 673 was great...
	good sesame flavor
	good flavor, easy to eat, a bit chewier than the others
	seaweed is too long. feels like I'm eating spaghetti. Would be an improved experience if the pieces were smaller.
	275 should be more greenish in color
	Sample 275 was the best out of the three for me. I liked the brighter color as well as the saltier flavor. It reminds me more of the seaweed my mom makes back home. It is slightly chewy the way seaweed should be and I enjoyed the texture
	The color for this sample is better than sample 479. Texture wise, they are pretty much similar.
	I wish sample 275 was a little firmer like 479, it's just a little too soft and chewy and its harder to get out of my teeth.
	275: The texture was too soft and there was not enough acidity
	275 tastes slightly less bitter than 673
	Had a weird aftertaste
	maybe a little more sesame oil.
	this sample feels slightly slippery, I don't like that
	Slightly bitter/off flavored but better texture and chewiness
	275 Texture was a little rubbery
	275; I can't tell the difference very well between this sample and 673, but I enjoy it a lot.
	Sample 275 was too difficult to chew.

	Sample 275 was more enjoyable than sample 673, the juiciness helped a lot and the firmness was amazing.
	I thought the sample had an interesting flavor but found the texture to be generally stringy and a little too chewy. I would have preferred a more crunchy sample similar to a lettuce.
	275 was a tad chewy, but the carrot and sesame added a nice flavor and crunch.
	275 had the best texture, it was slightly chewy, but more acceptable than the other samples. The flavor was also the right blend of dressing and sea vegetable.
	Texture reminds me a bit of pasta. Very nice!
	275 tastes okay but is too mushy, and also a little too chewy in some parts
	I'm a bit worried that I'll just like all of them so much I find it hard to give good feedback... I really like food in general :D Anyway, this is very good! It's not too sweet, which I like. I like having it more savory and sour like this. Very tasty. Nice texture too.
	275- I liked the crunchiness of this sample.
	Sample 275 was okay. The seaweed's texture was pretty soft, and the carrots overpowered the salad both in texture and in flavor. The major flavor I got from the salad was of carrot, with a hint of the dressing and seaweed.
	275 needs salt, I like the level of sweetness
	I thought the texture of sample 275 was perfect. It still had some crunch to it, but it was tender enough to chew easily. The taste was also very good. Not an overpowering seaweed taste.
	Enjoyed the flavor (tangy, almost nutty) more than 479!
	Sample 275 had a saltier flavor than the other two samples, and I think that brought out some other umami flavors going on in the seaweed. There were a few lighter green pieces in sample 275, which made me like the appearance of it more than the other two samples. I liked the texture of 275 because it seemed just right for chewiness. I thought 673 was OK, and I wasn't a big fan of 479 overall, but 275 was so good I made a point to finish the sample after answering the questions. On all three samples I felt a little anxious trying to get just the right amount on my fork; the pieces were long, and it was awkward to control how much I scooped or wrapped around my fork... I'm glad I wasn't trying to figure out how to eat it at a restaurant! I would order 275 again if it was served, and I would like to learn how to make it myself. Yum.

	257 (number on sample, not number below) - again the clumpiness and length were problematic, but as opposed to #479, the taste was better, no initial bitterness.
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Summary comments report for sugar kelp (seaweed) sauerkraut

Sample coded 762: 100% cabbage sauerkraut (control).

Sample coded 516: Blanched kelp/cabbage sauerkraut.

Sample coded 137: Blanched frozen kelp/cabbage sauerkraut.

Sample	Comment
100% cabbage sauerkraut	516 tasted better than 137 as it wasn't as fishy. 762 is just a traditional sauerkraut, however, i like the addition of seaweed because it adds another layer of texture.
	It was good crunchy fresh kraut except it was a little on the bland side.
	Overall I like this sample the best, but I can't tell if there is any seaweed in it. With samples 137 and 516, I could tell there was seaweed in the sample, albeit too much. I would definitely eat sample 762 regularly, but I wouldn't know there was seaweed in it (if there was).
	762 - perhaps TOO crunchy. Not as salty as the seaweed-cabbage combo krauts, but tangier (more acidic) Like the seaweed/cabbage combos better!
	tastes very vinegary.
	i like this one very much.. A great mixture of all the different taste mixed all together.. and you can taste all of them...
	For 762 I didn't notice any odor like I did with 137. This looked like cooked cabbage with no seaweed mixed in. Taste was also mild, not too salty.
	had more pronounced `funky` regular sauerkraut odor than the seaweed samples.
	very plain, fresh, and crunchy. Not as moist as most sauerkraut.
	762 was too bland, not salty enough sample was very crunchy seems that fermentation was not complete, sample was dry and lacked adequate moisture, cabbage pieces were too large.
	Tastes more like traditional sauerkraut, don't like that it is all one color (would not choose it over kraut mixed with dark seaweed based on appearance).
	Good.

	Much dryer than traditional sauerkraut I have enjoyed. Also saltier and less tangy.
	I like the texture of this sample (762) a lot, but I don't like its taste as much as the other two samples, 516 and 137. I like the oceany taste that seaweed adds.
	This sample (762) was strong in the salty/sour/tangy sense, with an obvious vinegar taste. I would eat it, but in small quantities, and probably to balance out another food.
	sample tasted good, the texture was a bit too chewy/crunchy for me milder taste then 137. Less salty but less flavorful.
	I like this sample more than 516 and 137, to me it has a more fresh clean taste. I like the texture.
	I liked this sample compared to other samples.
	762 it does have the good taste but the thing is if it would be little soft it would have been more soft.
	The taste of this sample (762) was better in my opinion than the other two (516 and 137) but i think it was just because it tasted more like a traditional kraut. The only negative thing I would say about it is that the texture was very crunchy and almost had a squeaking feeling against my teeth.
	762 tasted fairly normal, but was more firm and less tart than I expect kraut to be.
	Hit of vinegar, as expected with sauerkraut.
	Sample 762 has a lighter taste than I'm accustomed to. I would pair it with a dish containing applesauce; it is very light and sweet in flavor, compared to other sauerkraut (in my mind).
	Sample 762 was slightly less potent in flavor than samples 137 and 516. It was also less soggy/slimy.
	It's alright, but is a bit one dimensional. Sample 516 had much more variety in flavor and texture.
Blanched kelp/cabbage sauerkraut.	I think it's way too sour and it's got an off-flavor which is hard to describe. `Gasoline` is the only word I can think of now.
	I initially slightly liked the taste of sample 516. After answering and clicking next, I got an after taste I did not like. I would say I slightly dislike the taste.
	Very awkward to eat: sample wanted to come up in 1 big ball on my fork. A jarring contrast after 762. If I had to rate samples, I'd go 762, 137, add a few slots, and reluctantly add 516.
	It has a much cleaner flavor than 137. Less fishy.
	sample 516 had a bitter mineral-y taste that lingered, reminding me of a non-sodium salt.

	Sample 516 looks and smells good but the flavor is what knocks it down a lot.
	516 tasted pretty much the same as 137 except the flavor was not as strong - I would not buy or recomend 137 or 516.
	I liked the crunchy seaweed texture a lot.
	I think the seaweed flavor is too strong in this sample. It is even stronger (to me) than sample 137. I do like the texture and think it has a little more salt than sample 137 (which I like), but it is too fishy.
	I thought the seaweed consistency improved from sample 137 to sample 516. I found it much easier to get a reasonable bite out of sample 516 than I did out of sample 137.
	stronger briny smell than 137.
	Sample is very salt compared to 762. 762 was most comparable to traditional sauerkraut because it has a characteristic tanginess. Sample 137 and 516 are much saltier with 516 having a fishy flavor. 762 had the most mild base flavor with the exception of typical sauerkraut characteristics.
	Really tasty! Perfect texture.
	This sample (516) smells more like grass than the previous sample (762).
	The odor of this sample is mild, it was salty, but the seaweed was good. It had a good texture and a nice crunch when biting into it.
	a little too salty.
	Very tasty, but a little heavy on the amount of seaweed. Both samples with seaweed had a moister mouthfeel, which was nice, and not as dry as 762. This sample tasted a little saltier than 762 and 137, but I liked the saltier taste.
	516 was more salty and sour than 137 cabbage pieces were too large.
	Not noticing much of a difference between 137 and 516. I like 516 slightly more because it had slightly more of the vinegar flavor.
	Smelled like low tide.
	The texture of the seaweed compliments the crunch of the cabbage well.
	516 good but a bit salty.
	Too salty to swallow.
	This sample, 516, is a bit too vinegary for my taste, but I like the taste and the texture overall.
	I liked that this sample (516) was more well-rounded than sample 762, but my favorite is still definitely sample 137. This sample

	(516) had the pungent /sour/fizzy/vinegar flavor that sample 762 had, so the seaweed goodness didn't come through as well.
	Stronger seaweed flavor than 137. Good, but tasted more like seaweed than sauerkraut.
	It's ok, I feel it's too raw tasting as if it wasn't meant to be prepared for a consumer.
	516 has more sour in the taste but overall it was good.
	This sample (516) was sour and crunchy and refreshing.
	More liquidy than the first sample, made it taste more pickled. Enjoyed the slight sea taste.
	The saltiness and bitterness, and the contrast of sliminess and crunch, made me like sample 516 less than sample 762. I think if the flavor had been better, I wouldn't have minded the slimy texture as much.
	The smell is bit off-putting. It reminds me of a mud flat at low tide.
	I normally don't care for kraut, but this is pretty good!
Blanched frozen kelp/cabbage sauerkraut	I think more seaweed gives the sauerkraut a better texture.
	Slight bitter aftertaste. Also, I really wish I didn't get samples stuck in my teeth.
	137 had a big wad of seaweed clumped together which I found off-putting.
	It tasted like kraut with seaweed in it - the seaweed gave it a brackish, fishy taste.
	Compared to 516, 137 had more fishy smell and dryer texture (more crunchy).
	The texture and appearance of the sample are great, but the seaweed taste is a little too strong for my taste. This is why I disliked it overall.
	I found it difficult to take small portions of sample 137 when compared with sample 762. It seemed like the seaweed pieces may have been too long and got tangled together.
	lacks the tanginess that I enjoy about traditional cabbage based sauerkraut products.
	Compared to the 762 and 516, 137 seems to have a bitter after note. 516 was tasty with perfect crunch to it The color was interesting as well. 137 has overpowering seaweed and just a strand of cabbage here and there.
	Sample 137 still had a grass like smell like sample 516, but it was not as pungent in its smell.
	137 tasted quite salty to me, but I did like the crunch of the cabbage not as salty as 516 so it tasted better to me.

	This sample was a little more interesting than the cabbage only sample (762). The kelp added a nice oceany taste.
	Nice sample (137) some of the cabbage pieces were too large
	Sample 137 was a little on the salty side for me. I like more vinegary than salty.
	137 had a slightly better flavor than 516. Sample 516 had a slight slimy appearance and texture. Sample 137 tasted a little more saltier than 516, but 516 had a stronger briny/sea taste, which I did not like. The overall texture was good for both 516 and 137. I think if the sea vegetable was chopped into smaller pieces and incorporated into the kraut better, I would like 137 and 516 more.
	I enjoy this sample, though it also smells like low tide.
	it seemed like sample 137 had longer, stringier pieces of seaweed than 516. I think I liked the consistency of 516 better.
	This was my favorite of the three. I realized after moving forward that sample 762 also exhibited a sweetness. The current sample (137) had the best tangy flavor resembling sauerkraut.
	The taste and the texture of this sample, 137, are very similar to those of sample 516. I won't be able to tell them apart.
	These two items together are a weird combination but it's certainly interesting. 512 was the most fishy smelling and tasting. 137 appeared to have more seaweed than 512 but the flavor was much more balanced.
	This sample (137) smelled like fond memories of Maine beaches/coast and a particular lobster restaurant my family went to often in Harpswell when I was growing up. I really loved this at first bite. I'm OK with the slight metallic flavor because the seaweed really does some amazing stuff to balance out the flavors. I didn't get the pungent, sour & fizzy traits that I got from sample 762, and this sample (137) had a much richer and more complex flavor. Would eat this anytime!
	Too salty to eat a lot of, but tasty otherwise.
	I feel this is more balanced or mild than sample 516 although depending on taste.
	it was hard to get cabbage and seaweed in the same bite. also i had clumps of seaweed in my sample which effected how appetizing the sample appeared to me. sample 156 looks better mixed but i haven't tried it yet.
	137: Fishy and nauseating aroma. Not too intense but slightly repulsive. overall couldnt tell much difference between 516 and 137. 162 is good overall. Wouldnt tasting multiple samples at the same time counter intuitive??

	137 has the very good taste kinda like little sour in taste and little crunchy.
	I thought this sample tasted more fishy than 516 but it was still pretty good.
	Aroma is like the ocean (#137) The seaweed parts seem a bit mushy compared to the cabbage. Overall not bad.
	I did not feel much difference between sample 516 and 137, the smell was slightly different(in neither good nor bad way), 516 was a bit more sour.
	this one had a much stronger seawater/fish/seaweed flavor than 516 did.
	More salty, than pickley
	I liked sample 137 better than sample 516. It wasn't as salty tasting and the textures matched better together in my mind. Both sample 137 and 516 had ocean scents, but sample 137's scent wasn't as overpowering. Although I love the smell of the ocean, it can be an off-putting scent for my food.
	Samples 137 and 516 were indistinguishable to me.
	I like this one better than 516. It's a bit crunchier overall and the odor is not as strong. It definitely smells like the ocean but the smell is fresher.
	Trying all the samples side by side, I think 137 is my favorite. It has the most varied flavor. I love the texture of both seaweeds, and frankly would eat it for that alone. 762 seems pretty bland and basic. There is nothing wrong with it, but it is definitely not as interesting as 137 and 516.

APPENDIX I: Additional results and discussion on the physicochemical and microbial properties of kelp/cabbage used for sauerkraut (Chapter 3: Impact of blanching, freezing, and fermentation on physicochemical, microbial, and sensory quality of sugar kelp (*Saccharina latissima*))

Color properties of kelp for sauerkraut: Product form had no statistically significant effect on color, therefore data for whole blades and shredded slaw were pooled and analyzed together. Freezing significantly reduced the L* value and increased the b* value of fresh samples as compared to the other four fresh kelp treatments (Table I.1). Blanching also increased the L* values significantly as compared to raw samples in the fresh samples prior to mixing it with cabbage (1:1). A t-test analysis showed a significantly higher L* values when mixed with 50% cabbage as compared to raw kelp treatments only. Notably, only blanched frozen kelp/cabbage mix (blend) had a higher a* value as compared to frozen kelp only.

Table I.1: Color (Hunter L*, a*, b*) of raw (fresh) kelp or cabbage and kelp/cabbage blend treatments prior to salting [mean ± SD (n = 3)]

Treatments	L*		a*		b*	
	Raw (fresh) samples before mixing	Kelp/cabbage mix (blend) before salting	Raw (fresh) samples before mixing	Kelp/cabbage mix (blend) before salting	Raw (fresh) samples before mixing	Kelp/cabbage mix (blend) before salting
Cabbage only	71.0 ± 9.1 ^{aA}	71.0 ± 9.1 ^{aA}	0.2 ± 0.7 ^{cA}	0.2 ± 0.7 ^{bA}	20.6 ± 1.5 ^{aA}	20.6 ± 1.5 ^{aA}
Raw kelp and/or cabbage	17.4 ± 0.4 ^{cB}	51.0 ± 6.2 ^{bA}	2.7 ± 0.6 ^{aA}	1.9 ± 0.5 ^{aA}	11.6 ± 0.8 ^{cB}	17.2 ± 0.5 ^{bA}
Raw/frozen kelp and/or cabbage	16.4 ± 0.8 ^{cB}	49.7 ± 13.9 ^{bA}	2.2 ± 0.3 ^{abA}	2.0 ± 1.0 ^{aA}	17.0 ± 0.4 ^{abA}	12.1 ± 1.4 ^{cB}
Blanched kelp and/or cabbage	24.9 ± 0.5 ^{bB}	46.0 ± 11.1 ^{bA}	0.8 ± 0.4 ^{cA}	1.9 ± 0.3 ^{abA}	14.8 ± 2.1 ^{bcA}	11.3 ± 0.5 ^{cB}
Blanched/frozen kelp and/or cabbage	21.0 ± 0.1 ^{bB}	46.0 ± 4.6 ^{bA}	0.9 ± 0.3 ^{bcB}	1.5 ± 0.5 ^{abA}	13.5 ± 3.4 ^{bcA}	13.6 ± 1.2 ^{cA}

Only kelp or cabbage as fresh samples before mixing with 50% cabbage to form kelp/cabbage mixture prior to salting. Cabbage samples remained the same. One-way ANOVA among treatment (column); pairwise t-test before and after mixing with cabbage (row).

Superscripts: different small letters indicate significant differences among treatments (within column); different capital letters indicate a significant difference before and after mixing with 50% cabbage (within row). A probability level of 0.05 ($P \leq 0.05$) was selected for significance.

Hunter (L*, a*, b*): L* = lightness, a*= red/green, b*=yellow/blue.

Textural properties of kelp for sauerkraut: Mixing kelp treatments with cabbage increased the firmness significantly for the kelp/cabbage blend as compared to the fresh samples. Blanched treatments were significantly different from raw treatments in kelp/cabbage blend as compared to fresh samples before the mix. There were no significant differences observed among the fresh raw and blanched kelp treatments and it could be to the high standard deviation recorded in the samples.

Table I.2: Firmness (N) of raw (fresh) kelp or cabbage and kelp/cabbage blend treatments prior to salting [mean \pm SD (n = 3)]

Treatments	Firmness (N)	
	Raw (fresh) samples before mixing	Kelp/cabbage mix (blend) before salting
Cabbage only	301.0 \pm 14.4 ^{aA}	301.0 \pm 14.4 ^{aA}
Raw kelp and cabbage	160.1 \pm 22.5 ^{bB}	253.1 \pm 22.5 ^{abA}
Raw/frozen kelp and cabbage	169.4 \pm 27.1 ^{bB}	257.8 \pm 27.1 ^{abA}
Blanched kelp and cabbage	148.3 \pm 28.0 ^{bB}	217.3 \pm 28.0 ^{bA}
Blanched/frozen kelp and cabbage	151.7 \pm 22.5 ^{bB}	219.5 \pm 22.5 ^{bA}

One-way ANOVA among treatment (column); pairwise t-test before and after mixing with cabbage (row).

Superscripts: different small letters indicate significant differences among treatments (within column); different capital letters indicate a significant difference before and after mixing with 50% cabbage (within row). A probability level of 0.05 ($P \leq 0.05$) was selected for significance.

Microbial qualities of kelp for sauerkraut: There were no significant differences with fresh raw kelp treatments for both APC and fungi. However, mixing it with cabbage increased the APC and fungi counts except for raw/frozen and raw kelp treatments, respectfully. Presumptive *Vibrio* spp. was detected in two replicates of the raw (fresh) seaweeds and in the same replicates after mixing with cabbage. Interestingly, the presumptive *Vibrio* spp colony was detected in only one of the raw replicates after mixing treatments with 2% salt.

Table I.3: Microbial analysis of kelp or cabbage and kelp/cabbage blend treatments [mean \pm SD (n = 3)]

Treatments	APC		Fungi		Pathogen	
	Raw (fresh) samples before mixing	Kelp/cabbage mix (blend) before salting	Raw (fresh) samples before mixing	Kelp/cabbage mix (blend) before salting	Raw (fresh) samples before mixing	Kelp/cabbage mix (blend) before salting
Cabbage only	3.1 \pm 0.5 ^{aA}	3.1 \pm 0.5 ^{aA}	2.1 \pm 0.0 ^{aA}	2.1 \pm 0.0 ^{aA}	Absent	Absent
Raw kelp and cabbage	2.3 \pm 0.1 ^{bB}	3.1 \pm 0.5 ^{bA}	2.0 \pm 0.0 ^{bA}	2.0 \pm 0.1 ^{bA}	Present	Present
Raw/frozen kelp and cabbage	2.2 \pm 0.2 ^{bA}	3.0 \pm 1.1 ^{bA}	2.0 \pm 0.1 ^{bB}	2.1 \pm 0.1 ^{bA}	Absent	Absent
Blanched kelp and cabbage	2.1 \pm 0.1 ^{bB}	2.9 \pm 0.5 ^{bA}	2.0 \pm 0.0 ^{bB}	2.0 \pm 0.0 ^{bA}	Absent	Absent
Blanched/frozen kelp and cabbage	2.0 \pm 0.1 ^{bB}	2.7 \pm 0.5 ^{bA}	2.0 \pm 0.0 ^{bB}	2.1 \pm 0.1 ^{bA}	Absent	Absent

One-way ANOVA among treatment (column); pairwise t-test before and after mixing with cabbage (row).

Superscripts: different small letters indicate significant differences among treatments (within column); different capital letters indicate a significant difference before and after mixing with 50% cabbage (within row). A probability level of 0.05 ($P \leq 0.05$) was selected for significance.

CFU, coliform forming units; APC, aerobic plate count; Fungi, yeast and molds.

APPENDIX J: Linear regression for log reduction for all pathogen growth.

Processing *Vibrio* spp.....

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.954 ^a	0.91	0.908	0.37549

a Predictors: (Constant), Time, Product, Temperature

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Model	Regression	157.67	3	52.557	372.752	0.000b
1	Residual	15.51	110	0.141		
	Total	173.18	113			

a Dependent Variable: LogVibrio

b Predictors: (Constant), Time, Product, Temperature

Coefficients ^a						
Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	1.31	0.159		8.229	0.000
	Product	0.078	0.07	0.032	1.11	0.269
	Temperature	0.079	0.054	0.046	1.473	0.144
	Time	0.389	0.013	0.934	29.998	0.000

a Dependent Variable: LogVibrio

Processing *Escherichia coli*.....

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	0.931 ^a	0.867	0.864	0.43129

a Predictors: (Constant), Time, Product, Temperature

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Model	Regression	133.608	3	44.536	239.421	0.000b
1	Residual	20.462	110	0.186		
	Total	154.07	113			

a Dependent Variable: LogE.coli

b Predictors: (Constant), Time, Product, Temperature

Coefficients ^a						
Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	0.186	0.183		1.019	0.311
	Product	0.033	0.081	0.014	0.413	0.681
	Temperature	0.252	0.062	0.155	4.078	0.000
	Time	0.337	0.015	0.858	22.629	0.000

a Dependent Variable: LogE.coli

Processing *Listeria monocytogenes*.....

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.949 ^a	.901	.899	.38967

a Predictors: (Constant), Time, Product, Temperature

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Model	Regression	152.451	3	50.817	334.663	.000b
1	Residual	16.703	110	.152		
	Total	169.154	113			

a Dependent Variable: LogListeria

b Predictors: (Constant), Time, Product, Temperature

Coefficients ^a						
Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	1.933	.165		11.697	.000
	Product	.077	.073	.032	1.053	.295
	Temperature	-.246	.056	-.144	-4.410	.000
	Time	.410	.013	.997	30.497	.000

a Dependent Variable: LogListeria

Processing *Salmonella* spp.....

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.745 ^a	.555	.543	.45169

a Predictors: (Constant), Time, Product, Temperature

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Model	Regression	27.997	3	9.332	45.741	.000b
1	Residual	22.443	110	.204		
	Total	50.440	113			

a Dependent Variable: LogSalmonella

b Predictors: (Constant), Time, Product, Temperature

Coefficients ^a						
Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	1.480	.192		7.728	.000
	Product	.094	.085	.071	1.111	.269
	Temperature	-.058	.065	-.063	-.901	.370
	Time	.172	.016	.765	11.012	.000

a Dependent Variable: LogSalmonella

APPENDIX K: Preliminary study on thermal inactivation of *Listeria monocytogenes* on inoculated sugar kelp (*Saccharina latissima*)

Title

Effectiveness of blanching in reducing the populations of *Listeria monocytogenes* on inoculated sugar kelp (*Saccharina latissima*)

Introduction

Many technologies including blanching are used in the food industry to inactivate enzymes, reduce microorganisms, and preserve food quality. Blanching is a process in which the food product is exposed to hot water or steam for a short period of time. Blanching can reduce microbial growth and is easily accomplished in the food industry and at home. However, it is important to optimize blanching treatment as a kill step for, or to reduce, pathogens that may contaminate seaweed (sugar kelp) and cause illness when consumed. Pathogenic bacteria, including *Vibrio* spp., *Listeria monocytogenes*, and *Escherichia coli*, all pose a risk to sugar kelp safety. But *L. monocytogenes* was used in this study because it is ubiquitous in the environment, has a low infectious dose, and exhibits an increased heat resistance. Quantitative data for the eradication of *L. monocytogenes* in seaweed (sugar kelp) via blanching have yet to be reported, and could serve as a guideline for establishing safety regulations for the seaweed industry in the U.S.

Purpose

The purpose of this research was to assess the validity of blanching recommendations by determining the decimal reduction time (D-value) of *Listeria monocytogenes* inoculated onto sugar kelp.

Methods

Sixty grams of shredded sugar kelp was inoculated with 7.0 log CFU/g of *L. monocytogenes* (ATCC 19111, American Type Culture Collection, Manassas, VA), mixed together, transferred into 10.16 cm × 15.24 cm plastic bags (Ultrasource, Kansas, MO, USA) and sealed under 99% vacuum. Samples were placed in a 10 °C incubator for 45 minutes to get a uniform temperature in all samples before blanching. Pathogen population was evaluated as control after the 45 minutes post inoculation. Inoculated shredded kelp samples were subjected to blanching at three different treatment temperatures of 52 °C, 56 °C and 60 °C, and temperature treatment was processed in triplicate. Thermocouple (Omega, Stamford, CT) was inserted into the geometric center of the vacuum sealed bags containing uninoculated shredded kelp to record the come-up time for each temperature. Pathogen populations were analysed immediately at the come-up time and recorded as time 0 and then samples were analyzed periodically at 3, 6, 9, 12, 15, 18, 21 and 24 min after the come up time to evaluate inactivation of pathogens via blanching. Pathogens were enumerated in duplicates on PALCAM (Polymyxin acriflavine lithium chloride ceftazidime aesculin mannitol, EMD Millipore corporation, Billerica, MA) with a 5 ml tempered (50°C, Isotemp 105 water bath, Fischer Scientific, Dubuque, IA) soft brain heart infusion (BHI) agar overlay at 30 °C for 48 h to determine the effective log reductions of microbial populations.



Results

The average come-up times for samples blanched at 52 °C, 56 °C and 60 °C were 12 min, 16 min and 22 min, respectively. A z-value curve was not constructed because the D-values for *L. monocytogenes* inoculated on sugar kelp could not be determined experimentally at 52 °C, 56 °C and 60 °C (Figure K.1, K.2 and K.3). This is because it was difficult to get at least three pathogen populations points during blanching in order to determine the D-values and generate an equation from the slope. These preliminary results indicate a very rapid decrease in pathogen population shortly after the come-up time or no pathogen counts after the come-up time (Figure K.1, K.2 and K.3). Pasteurization, defined as a minimum five-log reduction in any bacterial pathogen could not be quantitatively achieved in sugar kelp at any of these temperatures since it was difficult to get D-values, and thus determine the z-values.

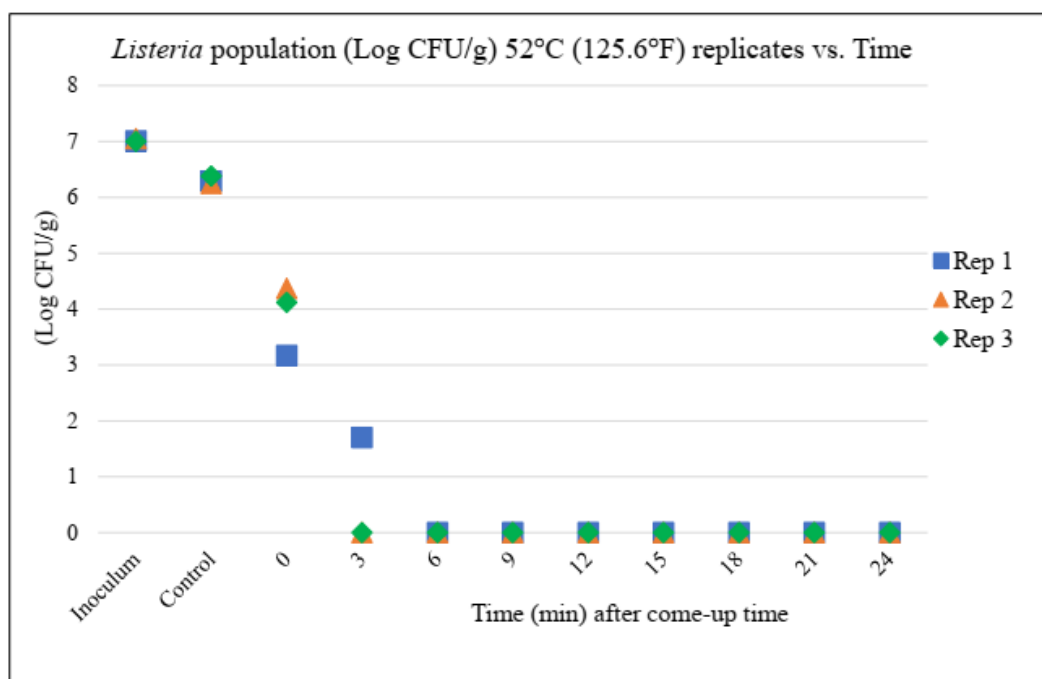


Figure K.1: Inactivation of *L. monocytogenes* inoculated on sugar kelp during blanching at 52 °C.

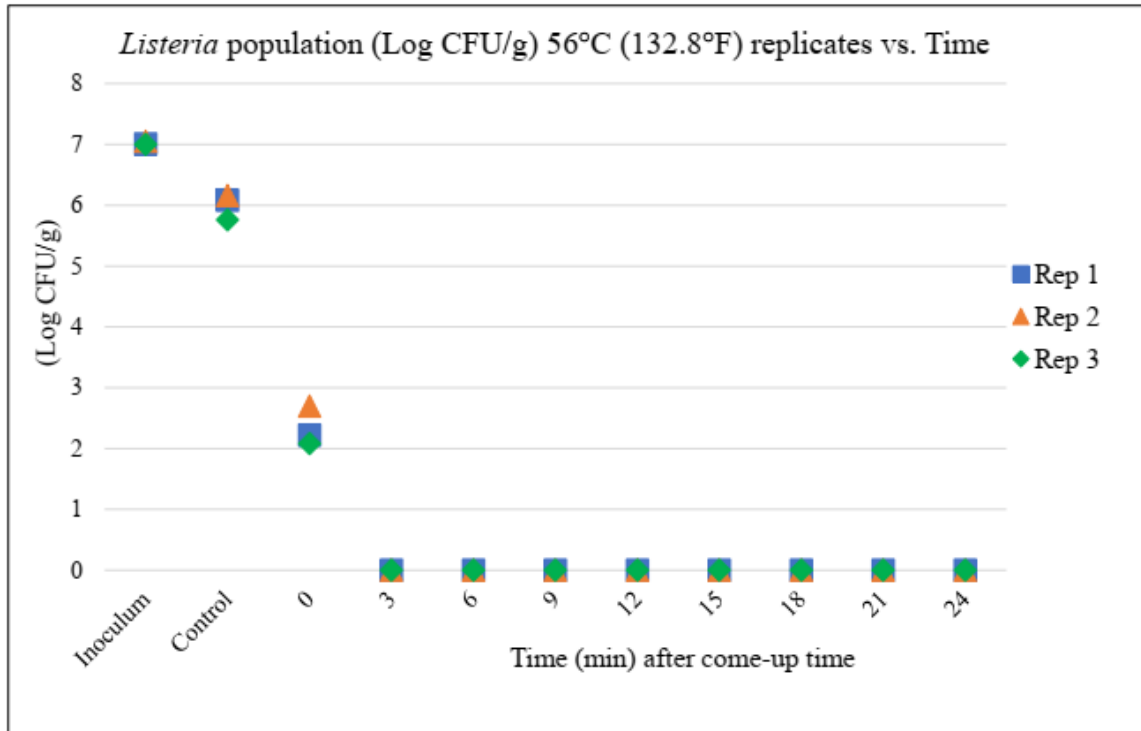


Figure K.2: Inactivation of *L. monocytogenes* inoculated on sugar kelp during blanching at 56 °C.

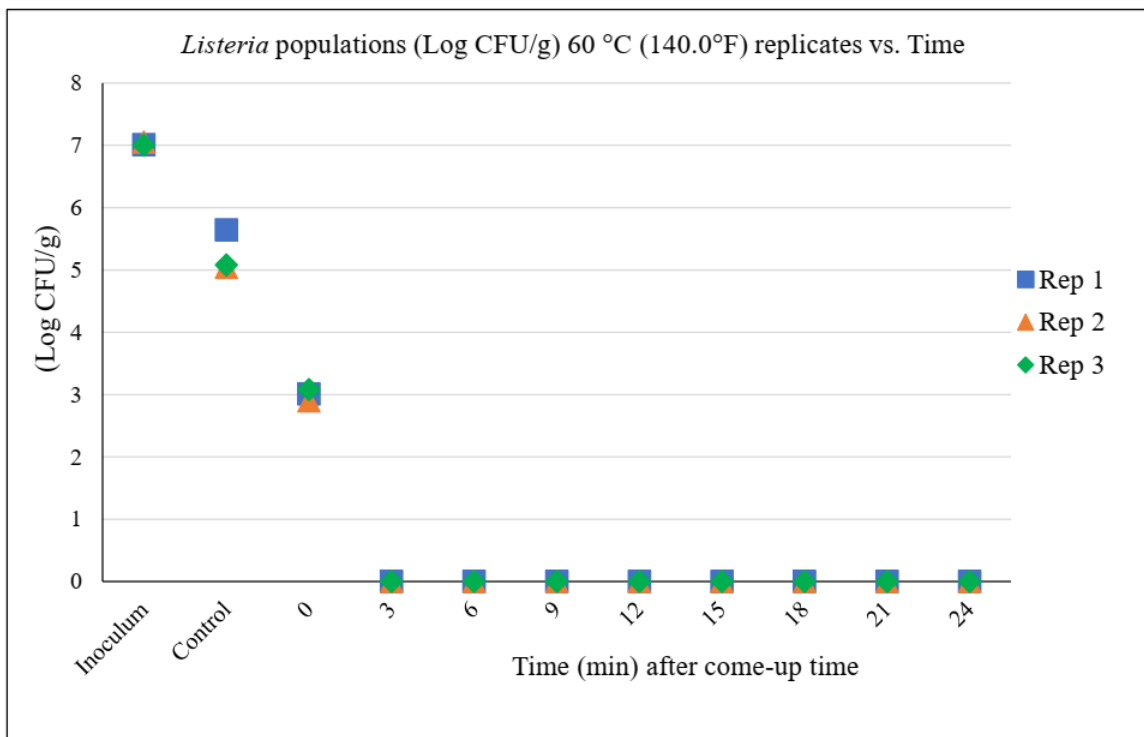


Figure K.3: Inactivation of *L. monocytogenes* inoculated on sugar kelp during blanching at 60 °C.

Significance

This pilot study serves as a guideline to inform future studies on the use of heat to inactivate bacterial pathogens, especially *Listeria monocytogenes*, to mitigate the risk of bacterial pathogen contamination in sugar kelp.

BIOGRAPHY OF THE AUTHOR

Samuel Akomea-Frempong was born in Ghana, Africa on April 4, 1990 and was raised up in New Tafo-Akim, Ghana. He attended Kwame Nkrumah University of Science and Technology for both his bachelor's degree in Agricultural Biotechnology in 2013 and master's degree in Food Science and Technology in 2016, which was in partnership with the Sokoine University of Agriculture (SUA), Tanzania, and the University of Copenhagen (KU), Denmark. He joined the School of Food and Agriculture at the University of Maine (United States) in 2018. Samuel is a candidate for the Doctor of Philosophy degree in Food and Nutrition Sciences from the University of Maine in May 2022.