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Development and Shelf Life Evaluation of a Novel Fermented Seaweed Sauerkraut Utilizing Commercially Important Maine Seaweeds

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DEVELOPMENT AND SHELF LIFE EVALUATION OF A NOVEL FERMENTED SEAWEED SAUERKRAUT UTILIZING COMMERCIALLY IMPORTANT MAINE SEAWEEDS

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An Abstract of the Thesis Presented
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Fermented vegetables can provide consumers with important health benefits, particularly due to the presence of probiotics. These fermented products have gained popularity with American consumers over the past decade. Therefore, a lacto-fermented seaweed sauerkraut, containing seaweed and cabbage, was developed to address this trend and to create a value-added seaweed product with extended refrigerated shelf life. The objective of this study was to evaluate the effects of kelp species and seaweed incorporation level on the fermentative success, microbial safety, consumer acceptability, and refrigerated shelf life of seaweed sauerkraut for 60 days post inoculation.

Six treatments with varying levels (25%, 50%, 75%) of farm-raised kelp (sugar kelp or winged kelp) were processed in triplicate. Shredded fresh kelp and cabbage were mixed with 2% kosher salt, inoculated with *Lactobacillus plantarum* (~10^6 CFU/g) and *Leuconostoc mesenteroides (*~10^1 CFU/g*)*, and fermented at ambient temperature until a pH of < 4.6 was achieved. The presence of pathogens (*Vibrio* spp.*, Salmonella, Staphylococcus aureus, Listeria* spp.) was evaluated and coliforms were enumerated in the fresh sauerkraut. Titratable acidity

(TA), pH, instrumental texture (shear force), color, antioxidant capacity, aerobic plate counts, lactic acid bacteria, yeasts and molds were measured periodically for 60 days after inoculation by the lactic acid bacteria (LAB). Additionally, acetic acid, lactic acid, sugar (glucose, fructose, sucrose), and ethanol levels were determined using high-performance liquid chromatography (HPLC). Multi-way analysis of variance was performed to evaluate significant ($p<0.05$) effects of the treatment variables. Sensory acceptability of these products was also evaluated to determine the consumer response to the 25% and 50% winged kelp and sugar kelp treatments.

Kelp species and incorporation levels significantly affected most variables tested in the freshly prepared sauerkraut. LAB grew fastest in the winged kelp treatments, with all products reaching a pH below 4.6 within 3 days and resulting in significantly lower pH and higher TA compared to the sugar kelp treatments. In contrast, the sugar kelp treatments did not achieve a pH of <4.6 until day 14. Kelp incorporation levels significantly influenced LAB counts with the highest average counts for both kelp species occurring in the 25% (7.5 log CFU/g) and 50% (7.7 log CFU/g) treatments. Notably, sugar kelp (SK) treatments had significantly lower shear force values than winged kelp (WK), and as concentrations of SK increased from 25% to 75% shear force decreased from 165.5 to 53.8 N. In regard to antioxidant capacity, there were no differences among SK and WK treatments but increasing seaweed concentrations improved total phenolic content and ferric reducing antioxidant power (FRAP) values of the sauerkraut. The SK treatments had higher levels of coliforms, and *Vibrio* sp. was detected only in the 75% SK treatment on day 7.

This study is the first to report on lactic acid fermentation to produce fresh seaweed sauerkraut. Seaweeds are primarily sold in dried form, and fermentation offers the potential to introduce refrigeration-stable, value-added, seaweed products to the market. Results indicate that seaweed sauerkrauts produced from fresh farm-raised kelp were refrigeration stable throughout the study.

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1. INTRODUCTION

1.1. Seaweeds

Seaweeds are marine macroalgae that are found in coastal areas around the world. Because they are found globally, there are over 10,000 species of seaweed and more that are continuing to be discovered (Mouritsen et al. 2013b). Many species can tolerate harsh environments that fluctuate in temperature, have rapid ocean currents, or are highly saline (Mouritsen et al. 2013b). Furthermore, seaweeds are usually found attached to hard substrates, because their environment is subject to strong waves. They have specialized tissues that serve as anchorage, called a holdfast, similar to a plant's root system (Mouritsen et al. 2013b). However, there are some species that float freely in the ocean. Typically, seaweeds grow in the intertidal zone because the sunlight helps them grow through photosynthesis, as all seaweeds contain chlorophyll and are photosynthetic (Mouritsen et al. 2013b). However, some species, like kelps, grow deep in the ocean.

Seaweeds are divided into three categories based on their color: Chlorophyta (green), Rhodophyta (red) and Phaeophyta (brown). The seaweed categories also differ in structural and biochemical features. Brown and red seaweeds are mostly found in marine waters, but green seaweeds can also be found in freshwater systems. There are approximately 6,200 red species, 1,800 green species, and 1,800 brown species of seaweed (Mouritsen et al. 2013b).

1.2. Sea vegetables

Seaweed can be used in a variety of applications including cosmetics, medicine, and food. Seaweeds used in foods are often called sea vegetables because any edible seaweed is termed a sea vegetable. Seaweeds are super foods that are rich in vitamins and provide important health benefits.

In addition, seaweeds can offer different benefits than land plants. For example, seaweeds can provide 10-20 times more minerals than land plants because seaweeds can obtain minerals from seawater (Makkar et al. 2016).

Seaweeds are rich in proteins and prebiotics, are good sources of bioactive compounds, are low in lipids, and contain non-starch polysaccharides (Fleurence, 1999). Furthermore, the polysaccharides found in seaweeds have the potential to have medicinal values for the body (Smit, 2004). Seaweeds are also a good source of fiber and the consumption of seaweeds may reduce the risk of colon cancer (Smit, 2004).

Brown, red, and green seaweeds offer different dietary benefits for humans. Red and green seaweeds are higher in protein and mineral content compared to brown seaweeds (Makkar et al. 2016). Red and green seaweeds contain ~50% and 30% protein content, respectively (Makkar et al. 2016). Nonetheless, brown seaweeds are rich in bioactive compounds such as phloroglucinol-based polyphenolic compounds, carotenoids, and polyunsaturated fatty acids (Holdt and Kraan, 2011). Bioactive compounds can influence human health and act similarly to antioxidants. Furthermore, brown seaweeds contain alginate, laminarin, and fucoidan which are resistant to human digestive enzymes, making them a source of dietary fiber (Charoensiddhi et al. 2016).

1.3. Seaweed industry

The seaweed industry is growing, and seaweeds that are used for direct human consumption around the world is a 5.29-billion-dollar (USD) industry (Chopin and Sawhney, 2009). This equates to 8.59 million tons of edible seaweeds harvested in a year, according to 2006 data (Chopin and Sawhney, 2009).

The worldwide seaweed industry farms approximately 220 seaweed species. However, the edible seaweed market consists of three dominant seaweed genera: *Laminaria* (or kombu), *Porphyra* (or nori), and *Undaria* (or wakame) (Chopin and Sawhney, 2009).

Asian countries are the largest consumers of seaweeds and most of the world's edible seaweeds are produced in Asian countries (Chopin and Sawhney, 2009). However, the recent radioactive contamination of Asian waters, caused by the leaking Fukushima nuclear plant in Japan, has created safety concerns about seaweeds from this area. Therefore, Maine's seaweed industry has the opportunity to grow to meet the increased demands for high quality seaweeds.

The Maine seaweed industry is the number one edible seaweed producer in the United States (NBC, 2015). In 2014, 17.7 million pounds of seaweed were collected by Maine harvesters and the number of Maine seaweed companies has doubled from 10 years ago (NBC, 2015). North American Kelp, Springtide Seaweed, Maine Coast Sea Vegetables, SOURCE Maine, VitaminSea, and Atlantic Holdfast Seaweed Company are just some of the more than 20 companies that grow or harvest seaweed in Maine. Furthermore, some of these companies have been around for 30 years or more (Maine Seaweed Council, 2016). One company, Maine Coast Sea Vegetables, harvests roughly 100,000 pounds of seaweed a year and sells their products to Amazon, Whole Foods, and other health food stores (Maine Coast Sea Vegetables, 2016).

The success of Maine's seaweed industry comes from the variety of edible seaweeds native to Maine's coast such as *Palmaria palmata* (or dulse) (Figure 1), *Alaria esculenta* (or winged kelp) (Figure 2), and *Saccharina latissima* (or sugar kelp) (Figure 3). Dulse are red seaweeds that are harvested from the early summer to fall (Maine Coast Sea Vegetables, 2016). Dulse is found in many food dishes across the world and is also commonly consumed as a snack. Dulse can also be used as a nutritional supplement because it is rich in iodine, protein, and iron

(Mouritsen et al. 2013a). Sugar kelp and winged kelp, are large brown seaweeds that have long and thin blades with wavy edges. There are about 300 different kinds of kelps that are classified under the laminariales, edible kelps (Mouritsen et al. 2013b). Kelps often create kelp forests in the ocean because of their large size that can reach up to 50 meters long (Mouritsen et al. 2013b).

Figure 1. *Palmaria palmata,* also known as dulse (McKirdy, 2015).

Figure 2. *Alaria esculenta,* also known as winged kelp (Norwegian Seaweeds, 2018).

Figure 3. *Saccharina latissima,* also known as sugar kelp (Algolesko, 2018).

The winged kelps and sugar kelps found in Maine offer consumers important health benefits. The winged kelps are a good source of Vitamin A (Mouritsen et al. 2013b) and protein, while sugar kelps offer medicinal and unique flavor characteristics to consumers. Sugar kelps are unique because they have a sweeter taste (Mouritsen et al. 2013b). When sugar kelps are dried, they excrete polysaccharides and mannitol, the compound responsible for the sweet taste of this seaweed. Overall, kelps are rich in vitamins, minerals, and phytonutrients (Mouritsen et al. 2013b). Some minerals found in kelps are calcium, potassium, iodine, and magnesium (Mouritsen et al. 2013b). In addition, kelps have a naturally high monosodium glutamate (MSG) content, responsible for their umami taste (Mouritsen et al. 2013b). Therefore, kelp is often incorporated in a variety of foods such as, soup, salad, cooked dishes, or sprinkled on food, like a spice. However, some varieties of kelps are better for human consumption because some are thin and soft, while others can be undesirably thick and tough.

While many of Maine's seaweeds are freshly harvested, few seaweeds are sold fresh to the market. Dried seaweeds have a longer shelf life than fresh seaweeds, therefore, many edible

seaweeds are sold in either dried or frozen forms. One potential alternative to drying seaweed is to ferment it. Fermenting could transform fresh seaweed into a shelf stable product. Furthermore, seaweeds are super foods that are rich in vitamins and provide important health benefits. Fermenting seaweed could increase its health benefits and create a non-dairy alternative probiotic product for consumers.

1.4. Lacto-fermentation

Fermentation can be described as respiration without air, meaning that microbial enzymes can cause chemical changes in food anaerobically. In addition, fermentation is a process in which an organism converts a carbohydrate into an alcohol or an acid. One type of fermentation is called lacto-fermentation, during which lactic acid bacteria convert carbohydrates, such as glucose, from fruits and vegetables into lactic acid (Paramithiotis, 2017).

The natural presence of lactic acid bacteria on vegetables allows for spontaneous fermentation to occur yet, using a starter culture in fermentation provides a more reliable and consistent fermentation (McFeeters, 2004). One of the most common starter cultures used in lacto-fermented products is *Lactobacillus plantarum* (Molin, 2001). However, there are many different species of lactic acid bacteria that are found in fermented products. *Lactobacillus, Leuconostoc, Pediococcus* and *Streptococcus* are the main genera involved in desirable food fermentations (FAO, 1998). Lactic acid bacteria are gram positive, aerotolerant, anaerobic, micro-aerophillic, bacteria. Lactic acid bacteria can be heterofermentors or homofermentors. Heterofermentors produce lactic acid, acetic acid and other products, while homofermentors produce mostly lactic acid. *Lactobacillus* bacteria are unique because some are heterofermenters or homofermenters (FAO, 1998).

The success of lactic acid bacteria growth is dependent on temperature, salt content, and oxygen availability (FAO, 1998). Furthermore, lactic acid bacteria growth can also be affected by the carbohydrate source and concentration and pH levels (Gupta et al. 2011). Changing the pH of the environment can affect how lactic acid bacteria inhibit other bacteria (Akbar et al. 2016).

1.5. Food industry uses for lacto-fermentation

One current food trend is that consumers are looking for more simple food products that contain fewer ingredients (Inova Market Insights, 2018). Simple products are considered by many consumers as healthier and more natural. One example of making products simpler could be to replace common artificial preservatives found in foods with food fermentation, which is a natural preservation process. One outcome of this trend is the increased development of lacto-fermented foods. Innova Market Insights reports that fermented foods and beverages increased by 35 percent in the U.S. from 2015 to 2016. Therefore, there is a lot of opportunity for new fermented products because the demand for fermented products will likely continue to increase in the near future.

1.5.1. Preservation

One of the reasons lacto-fermentation is used in the food industry is because of its ability to preserve vegetables, increase their shelf life, and enhance safety of food products. The process of fermentation preserves food naturally by decreasing pH and slowing the growth of spoilage bacteria. Fermentation utilizes the sugars in the product/vegetable and produces organic compounds, like propionic acid and lactic acid (Paramithiotis, 2017). The production of these acids creates an unsuitable (low pH) environment, causing antimicrobial activity.

Using lacto-fermentation for food preservation can also be termed as biopreservation. Biopreservation refers to the use of living microbes to extend the shelf life of foods (Akbar et al. 2016). Biopreservation is a natural method that decreases the use of preservatives or antimicrobials in foods, ingredients that consumers are avoiding. Lactic acid bacteria provide biopreservation because they produce lactic acid, acetic acid, hydrogen peroxide and other antimicrobial compounds, that help extend the shelf life of vegetables (Akbar and Anal, 2014). Additionally, lactic acid bacteria have antagonistic metabolites and bacteriocins (Akbar et al. 2016). Bacteriocins are natural proteins that have antimicrobial properties that can inhibit the growth of other similar bacteria or unwanted microbes, such as spoilage bacteria and pathogens (Akbar et al. 2016). Lacto-fermentation also helps to preserve antioxidants such as ascorbic acid, phenols, and colored pigments like beta carotene and anthocyanin (Panda et al. 2009). It is for these reasons that lactic acid bacteria improve the safety, shelf life, and quality of food products and are used in preservation.

Lactic acid bacteria are used for food preservation because of their ability to decrease food contamination by foodborne pathogens and spoilage bacteria. Lactic acid bacteria, particularly *L. plantarum,* helped to inhibit growth of foodborne pathogens (*Staphylococcus aureus, Salmonella* spp., *Clostridium perfringens, Bacillus cereus)* in a fermented red seaweed beverage (Ratanburee et al. 2011, Hayisame-ae et al. 2014). *L. plantarum* was reported to have the most promising ability to control fungi such as yeast and mold in fermented plant beverages (Prachyakij et al. 2008). Prachyakij et al. (2008) studied samples of fermented plant beverages that are common to Thailand. Some samples were contaminated with yeast and a total of 72 lactic acid bacteria strains were studied to see which strain could inhibit yeast contamination. They found that mold and yeast spoilage were less likely to occur in a fermented product using

L. *plantarum* as the inoculate species. However, more research needs to be done to determine why this inoculate had the best inhibiting effect.

1.5.2. Flavor

More commonly, lacto-fermentation is used to create food products with unique flavor profiles. Some examples of lacto-fermented products are sauerkraut, yogurt, kimchi, sourdough bread, fish sauce, and kombucha. Lactic acid bacteria are commonly used as starter cultures because of the role they play in providing flavor and texture to fermented foods.

Lactic acid bacteria are responsible for creating a unique sour taste because these organisms produce lactic acid, acetic acid, and hydrogen peroxide during fermentation. However, the LAB strain can influence the flavor of the product. Dongmo et al. (2017) compared the aroma composition and sensory profile of fermented beverages that were each produced with a different strain of lactic acid bacteria. Aroma plays an important role in how the flavor of a product is perceived. The results showed that different lactic acid bacteria significantly affected the aroma of the product and different lactic acid bacteria strains produced more aroma compounds in the beverages. More specifically, the *L. plantarum* strain was correlated with producing a fruity flavor.

The consumer acceptance of aroma, appearance, texture, and flavor of products with probiotics was tested by Luckow and Delahunty (2004) using blackcurrant juices. A blackcurrant juice that contained *Lactobacillus plantarum* was compared to seven conventional blackcurrant juices that were popular in the market. Consumers described the probiotic drink as perfumey in aroma and savory and sour in taste (Luckow and Delahunty 2004). This research shows that probiotics can provide a detectable flavor difference in drinks. The authors also determined that age of the panel members influenced the acceptance of probiotic juices, with older consumers

being more accepting of the probiotic drink. This is possibly because older consumers could be less sensitive to the unique flavor of the probiotic drink. In addition, females that were over 40 preferred the flavor of the probiotic juices and indicated that they would also drink them more frequently than conventional juices (Luckow and Delahunty, 2004).

Overall, lacto-fermentation can impact the flavor of products. There are several variables in a fermented product that can influence its taste. The overall acidity, salt, addition of spices, different microbial strains, and how the product is packaged, are a few examples of how flavor can easily be influenced.

1.5.3. Health

Consumers are becoming more interested in their personal health and are more concerned about making responsible food choices. It is for this reason that consumers are interested in foods that offer health or probiotic benefits (Granato et al. 2010). Foods that provide these benefits are termed functional foods. Lacto-fermented products are considered functional foods because lacto-fermented products provide consumers with important health benefits, particularly probiotic benefits. Overall, probiotics are "good" bacteria that administer health benefits, like disease prevention or improved digestion (Dunne et al. 2001; Luckow and Delahunty, 2004). Beganovic et al. (2011) defined a probiotic as containing 10^6 CFU/g live probiotic strains. Probiotics aid in disease prevention because they compete with pathogens (e.g. *E. coli, Salmonella* spp.*)* by binding to epithelial cell binding sites to inhibit colonization by pathogenic bacteria (Akbar et al. 2016). In addition, some probiotics can produce bioactive compounds that inhibit the growth of other bacteria (Akbar and Anal, 2014). Probiotics can aid in digestion because they can survive extreme conditions, such as wide pH ranges and can tolerate bile salts to ensure that they work in the intestinal passage of the body (Dunne et al. 2001). Some lactic

acid bacteria can offer probiotic benefits because of their strong ability to survive in the human gut and inhibit microbes. It can take the human body three hours to digest certain meals and this means that lactic acid bacteria must survive in the acidic conditions of the GI tract to administer health benefits to the body (Olejnik et al. 2005). Duangjitcharoen et al. (2009) studied the probiotic characteristics of *Lactobacillus plantarum* by researching how it could survive in the gastrointestinal tract. The results showed that *L. plantarum* was a successful probiotic because it survived in a range of differing environments. Furthermore, *L. plantarum* is commonly found in the human gastrointestinal (GI) tract. The human GI tract is an acidic environment that contains bile salts. These conditions are like the environment in a fermenting process and is why these bacteria can survive in that environment. In addition, this study confirmed that *L. plantarum* was safe to use in fermented products after studying mice that had consumed *L. plantarum* fermented products.

Consumers are very interested in products that contain probiotics. Foods and supplements containing probiotic ingredients were worth $16 billion (USD) in 2008 and the market continues to grow (Granato et al. 2010). Interestingly, most probiotic products are dairy products, with yogurt being the most consumed product (Granato et al. 2010). Dairy products are not always ideal, due to lactose intolerance and specific diets (veganism) that preclude their consumption. Non-dairy examples of probiotics include supplements, sauerkraut, and kombucha. Therefore, there appears to be a lot of potential for the continued development of some non-dairy probiotic products.

1.6. Vegetable lacto-fermentation

1.6.1. Sauerkraut

Sauerkraut is a fermented cabbage product that is usually served as a side dish or condiment. Sauerkraut typically ferments from naturally present lactic acid bacteria. The microflora found on cabbage changes based on growing region, season, and cultivation patterns (Xiong et al. 2012). Sauerkraut typically contains cabbage and sometimes a mixture of spices and garlic. Cabbage is usually immersed in a 6-8% (w/v) salt solution and left at room temperature to ferment for 6-12 days in a mason jar (Xiong et al. 2012.)

It is important to have an anaerobic environment during cabbage fermentation. The sauerkraut should be kept lidded and the contents should be covered with enough brine to reduce the risk of oxygen exposure. Oxygen can increase the growth of other microorganisms that thrive in aerobic environments and this can spoil the fermentation and negatively impact the flavor of the product (Paramithiotis, 2017).

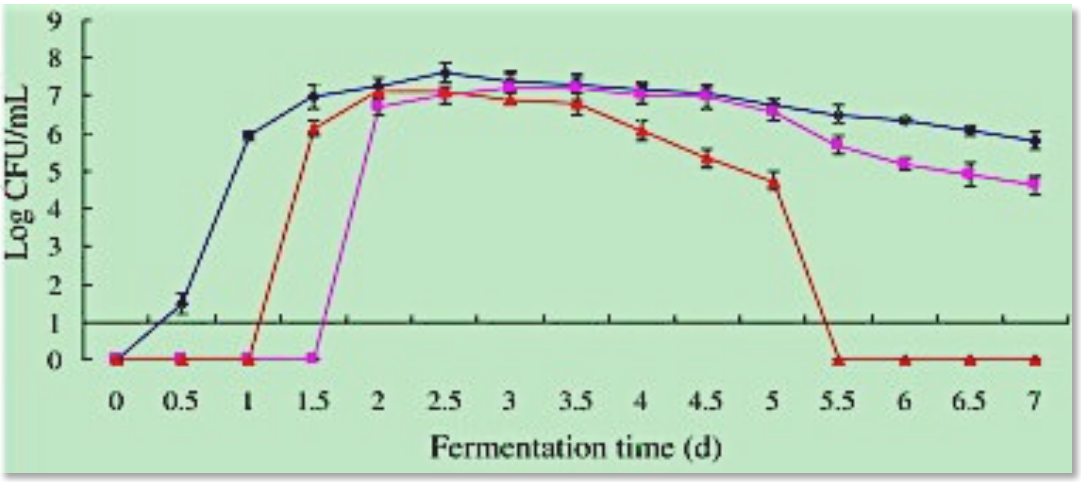
The addition of salt to the cabbage plays an important role in fermentation success. Salt helps improve the flavor of fermented products and, to some extent, inhibits growth of some spoilage bacteria (Xiong et al. 2016). Mainly, salt is used because it is responsible for maintaining the texture of the cabbage, as it prevents the cabbage from softening (Paramithiotis, 2017). Contrastingly, too much salt can negatively impact the fermentation. Xiong et al. (2016) found that high salt concentration (8% w/v) decreased the growth of lactic acid bacteria populations and delayed the fermentation.

The natural bacteria present in the sauerkraut changes throughout the fermentation time and influences the outcome of the fermented product. The bacteria *L. plantarum*, *L. casei* and *L. zeae* are dominant species found in sauerkraut. In a study by Xiong et al. (2012), *L. plantarum*

reached its maximum concentration on the second day and then decreased throughout the seven-day period, while *L. casei* reached its maximum on the third day (Figure 4). *L. zeae* survived the shortest amount of time because it is most sensitive to high acidity (Xiong et al. 2012). *L. mesenteroides* was the dominant species at the beginning of the fermentation, while *E. faecalis*, *L. lactis*, *L. zeae*, *L. plantarum* and *L. casei* were present towards the end of the fermentation period (Xiong et al. 2012).

In sum, the population dynamic of spontaneous sauerkraut production changes throughout the fermentation period. Knowledge of the population dynamic of microflora in sauerkraut can help control the fermentation process and how to use starter cultures to provide a more consistent population dynamic.

Figure 4. Changes in the flora of *Lactobacilli* during Chinese sauerkraut fermentation.



(◆ *L. plantarum*; ■ *L. casei*; ▲ *L. zeae*) ($n = 6$). Values below 1 indicate that the count was less than the detection limit (10 CFU/mL). Obtained from Xiong et al. (2012).

Spontaneous fermentations are highly dependent on the lactic acid bacteria present and this results in unpredictable fermentations. If a spontaneous fermentation does not have enough lactic acid bacteria present from the start this can affect the acidity levels of the treatment and

risk spoilage of the product (Paramithiotis, 2017). The use of starter cultures in cabbage has contributed to more uniform ferments and improved fermentative function. Xiong et al. (2014) tested four different lactic acid bacteria strains and found that *Leuconostoc mesenteriodes* grew faster than the other strains and produced lactic acid sooner. The results also indicated that different LAB strains had varying tolerances to acid. The addition of starter cultures to the sauerkraut can also improve the texture of sauerkraut. Johanningsmeier et al. (2007) tested sauerkraut with and without starter cultures added and found that the firmness in spontaneous fermentations varied among treatments and decreased over time. Yet, the addition of L. *mesenteroides*, a lactic acid bacteria starter culture, to the sauerkraut resulted in better texture. Therefore, adding a starter culture to a fermentation can influence the properties of the fermented product.

1.7. Seaweed fermentation

The application and possibility of fermenting seaweed is a relatively new concept, as most of the fermented products available consist of terrestrial foods. Although recipes for seaweed flavored sauerkrauts are available on the internet and seaweed products exist in the market, they consist primarily of cabbage and contain extremely small amounts of seaweed, around one tablespoon per cabbage head, likely added for visual appeal and some flavor notes. We have not found a sauerkraut containing significant levels of seaweed, or any peer reviewed paper on this topic in the scientific literature. One reason for this is that seaweeds are difficult to ferment, making this a topic in need of further research. Seaweeds contain polysaccharides that are not ideal fermentation substrates (Uchida and Miyoshi, 2013). For example, major polysaccharide components in brown algae include alginate and fucoidan. Alginate structure is made of guluronic and mannuronic acids (Marcel and Meyer, 2013). Fucodians have structural

units that consist of fucan and sulfated polysaccharides (Marcel and Meyer, 2013). Red algae contain agar and carrageenan. Green algae and seagrasses contain cellulose and hemicellulose as major components (Uchida and Miyoshi, 2013). However, seaweeds also contain sugars that could possibly be utilized during fermentation. Hwang et al. (2011) compared lactic acid yields from seaweed sugars to land plant sugars. The results showed that seaweed contained diverse sugars (D-galactose, D-mannitol, L-rhamnose, D-glucuronic acid, and L-fucose) and the predicted lactic acid yields were comparable to fermenting land plants. Furthermore, recent studies have reported successful fermentation of brown and red seaweeds by using lactic acid bacteria starter cultures and various seaweed pre-treatments.

Gupta et al. (2011) evaluated the effects of thermal processing of seaweed and aerobic/anaerobic conditions of seaweeds on the growth of lactic acid bacteria. The fermentative capability of three Irish brown seaweeds (*Himanthalia elongata*, *Laminaria digitata*, and *Laminaria saccharina)* were tested by using *L. plantarum* as the starter culture. For the heat treatment, seaweeds were placed in an autoclave at 95º C for 15 min. The results from this preliminary study suggested that growth of lactic acid bacteria could not be sustained in any of the raw seaweed species, however lactic acid bacteria growth did occur in the heat treated *L. digitata* and *L. saccharina*. The heat treatment resulted in an increase in the amount of sugars readily used by *L. plantarum*. Furthermore, heat treatment could help reduce the microbial surface load on the seaweed and increase availability of nutrients and sugars in the seaweeds for the bacteria (Gupta et al. 2011). Overall, this study showed that heat treated *L. digitata* and *L. saccharina* were suitable substrates for lactic acid fermentation by *L. plantarum*.

The results showed the highest cell populations were found in the *L. digitata* treatments, while *L. saccharina* achieved a faster fermentation time because it took less time for maximum cell growth to occur (Gupta et al. 2011).

Gupta et al. (2011) also tested the effects of aeration conditions on lactic acid bacteria growth and acetic acid production by changing the speed of agitation. Microaerophillic and aerobic conditions were tested and growth kinetics, such as the lag period and maximum specific growth rate of the *L. plantarum*, were measured. The agitation speed of the culture influenced the lactic acid bacteria growth; as the agitation speed was increased, the maximum cell growth decreased. This was due to the different metabolic processes that occurred in the presence of oxygen during fermentation (Gupta et al. 2011).

Ratanaburee et al. (2011) studied the use of *L. plantarum* as a seaweed starter culture by researching the optimal conditions and ingredients for producing a functional fermented red seaweed beverage. Dried *Gracilaria fisheri*, a red seaweed that is commonly found in Thailand, was used in this study because it contains carotenoids, polyphenols, and phenolic acid that can be beneficial for human health (Ratanaburee et al. 2011). The authors studied four different fermented plant beverage formulations. The first formulation, treatment A, consisted of red seaweed, sucrose, and water. Treatment B was similar to treatment A, but had the addition of a 5% starter culture of *L. plantarum*. Treatment C was also similar to treatment A, however it included an increased sucrose concentration and the addition of monosodium glutamate (MSG). Treatment D was the same as treatment C, but had the addition of a 5% starter culture of *L. plantarum*.

Gamma-aminobutyric acid (GABA) is often found in fermented foods because some lactic acid bacteria (*Lactobacillus brevis, L. plantarum* and *Lactococcus lactis*) can produce it. GABA has been shown to prevent diabetes, inhibit the growth of cancer cells, and reduce hypertension (Adeghate and Ponery, 2002). GABA is a non-protein amino acid compound that is made through the decarboxylation of glutamic acid by the glutamate decarboxylase enzyme (Siragusa et al. 2007). Because of this process, MSG was added to treatments C and D to determine if it could lead to an increase in the amount of GABA synthesized (Ratanaburee et al. 2011). Results indicated that the treatments that were inoculated with a starter culture had a higher number of beneficial microbes, total bacteria count (TBC), and LAB count after the fermentation period of 60 days. Additionally, LAB was the dominant bacteria present in all treatments. Treatments C and D, the treatments with the added MSG, produced higher amounts of GABA during the fermentation period, with treatment D producing the highest amount. However, the MSG treatments scored the lowest on a sensory test that measured flavor, odor, and color due to their salty taste. Lastly, as the sugar content decreased during the fermentation the acidity level increased.

Ratanburee et al. (2011) showed that fermentation of dried *Gracilaria fisheri* is possible. However, the balance between having a palatable product and having a high GABA content still remains in question. Adding MSG to seaweed fermentations resulted in an unpalatable product, despite the high GABA production. This research also showed that yeast contamination can be a problem during seaweed fermentation, as yeast contamination was found in all treatments.

Research by Uchida et al. (2007) on the fermentation of the brown seaweed, *Undaria pinnatifida,* addressed the problem of bacterial contamination in fermentation by determining how lactic acid bacteria could regulate the growth of contaminant bacteria. This research also

provided alternatives to the need to sterilize seaweed prior to fermentation. Sterilizing seaweed can reduce the load of undesirable microbes during fermentation, however, sterilizing can destroy some of the nutritional properties and the appearance of seaweeds. The appropriate inoculate species or salt content could help to reduce the microbial load and omit the need to sterilize seaweed prior to fermentations.

1.7.1. Inoculate species

Uchida et al. (2007) studied fourteen different lactic acid bacteria strains to determine which strain could reduce the presence of spoilage bacteria in fermentations. All the treatments that had no added inoculate spoiled, indicating that the addition of an inoculate may be a necessary component to successful seaweed fermentation. The inoculates *L. brevis*, *L. plantarum*, *L. casei,* and *L. rhamnosus* showed the highest inhibition of bacterial contamination and showed 90% predominance in the cultures (Uchida et al. 2007). These inoculate species, except for *L. brevis,* were homofermenters and they all produced the highest quantity of lactic acid compared to the other strains. The high acid production by these species lowered the pH of the fermented culture, most likely causing the successful inhibition of contaminant species.

The temperature (21° C) used during this study could have played a role in the inhibition capabilities of certain strains. Typically, cultures are incubated at 37° C for optimal growth. However, this experiment was conducted at a temperature closer to the natural temperature of the ocean and the environment of seaweed. Because of this, LAB strains that showed the highest populations in these conditions could be used to ferment seaweed (Uchida et al. 2007).

1.7.2. Salt content

Uchida et al. (2007) also researched the conditions for fermenting seaweeds by investigating the effects of salt content, a variable that had never been tested before. Treatments

were made using wakame (*Undaria pinnatifida*) seaweed powder, water, 3.5% salt (w/v), inoculate, and cellulase. Similar mixtures were made without the addition of salt. Cellulase was added as a pretreatment for the seaweed powder because previous findings by Uchida et al. (2007) showed a higher soluble sugar content and more successful ferment with the addition of cellulase. Cellulase allows for saccharification, the hydrolysis of polysaccharides to soluble sugars to help support fermentation. Cellulase also helped to avoid rotting during fermentation (Uchida et al. 2007).

The presence of salt helped to inhibit unwanted microbes, as cultures without salt grew contaminant bacteria that spoiled the fermented product. The concentration of salt added also affected the growth of *Lactobacillu*s starter cultures. *Lactobacillus* are not halotolerant and their growth is restricted as the salt concentration is increased above 5%. It was found that salt concentrations that ranged from 2.5-3.5% resulted in the most ideal fermentation conditions (Uchida et al. 2007).

1.7.3. Fermentation time

The effects of fermentation period on seaweed were determined based on the production of lactic acid, total acid, sugar consumption, and pH levels (Ratanburee et al. 2011). The authors measured the fermentation of a red seaweed, *Gracilaria fisheri*, throughout the course of 60 days and found that most biochemical changes occurred within the first 7 days. It was found that the highest levels of lactic acid bacteria occurred within the first ten days of the fermentation period with a gradual decline thereafter. The total bacteria count displayed a similar trend, where almost all treatments showed the highest count within ten days. In addition, the total sugar decreased the most rapidly after 7 days, this also resulted in the increase of total acidity. The pH of the

fermentation decreased the most rapidly within the first day of the fermentation. After 60 days, the pH in the tested treatments ranged from 3.2-3.8, with the initial pH range being 5-7.

In addition, similar findings by Uchida et al. (2007) determined that fermentation period of a brown seaweed, *Unidaria pinnatifida,* was seven days based on lactic acid production, pH consistency, and the increase in microbial counts. The microbial counts increased from the first day to the fourth or sometimes until the seventh day of the study. After this time, the microbial count gradually decreased while the pH stayed constant.

1.8. Justification

Maine's seaweed industry is growing and there are limited types of products available, creating a need for product development. Fermentation can be used to create a value-added refrigerated product that utilizes fresh seaweed and serves as an alternative to dried products. Fermentation could also be a low cost preservation method for small producers. Furthermore, fermenting seaweed could create a non-dairy alternative probiotic product for consumers, as well as increase the health potential of this super food that is already rich in nutrients.

Based on this review of the literature, the key variables that influence seaweed fermentation are salt content, environmental oxygen levels, inoculate species, temperature, and the pre-treatment of seaweed. At this point, seaweed fermentation is a relatively new concept and has mostly been tested in the development of fermented seaweed beverages, primarily for Asian consumers. While some of these variables have been studied on seaweeds, none have been tested on sugar kelp (*Saccharina latissima)* and winged kelp (*Alaria esculenta*), raising the question of whether these economically important Maine seaweeds can successfully be fermented. In addition, although seaweed sauerkraut recipes are available, to our knowledge, there are no scientific reports on the development of lactic acid fermented seaweed sauerkraut.

Additionally, the fact that seaweed sauerkraut recipes exist indicates that there is consumer interest in these types of products. Therefore, the overall objectives of this research were as follows:

1.9. Objectives

Objective 1: Develop novel seaweed sauerkraut products using a lactic acid bacteria starter culture and assess their sensory acceptability by a consumer panel.

Objective 2: Evaluate the effects of seaweed species (sugar kelp and winged kelp) and seaweed to cabbage ratios (75:25, 50:50, 25:75) on fermentation success, microbial safety, and physicochemical properties of seaweed sauerkraut under refrigerated (3° C) storage.

2. MATERIALS AND METHODS

2.1. Experimental design

The overall objectives of this research were to: 1) Develop novel seaweed sauerkraut products using a lactic acid bacteria starter culture and assess their sensory acceptability by a consumer panel, and 2) Evaluate the effects of seaweed species (Factor 1: sugar kelp, winged kelp) and seaweed to cabbage ratios (Factor 2: 75:25, 50:50, 25:75) on fermentation success, microbial safety, and physicochemical properties of seaweed sauerkraut under refrigerated (3° C) storage. Six treatments with varying fresh kelp (sugar kelp or winged kelp) levels (25%, 50%, 75%) were prepared in triplicate for a 2 x 3 complete randomized design with repeated measurements over time. Shredded kelp and cabbage were mixed with 2% kosher salt, inoculated with *Lactobacillus plantarum* (~10^6 CFU/g) and *Leuconostoc mesenteroides (*~10^1 CFU/g*)*, and allowed to ferment at ambient temperature (21-22° C) until a pH of < 4.6 was achieved. Subsequently, the sauerkraut was stored at 3° C and shelf life was evaluated. Fermentation was evaluated by measuring aerobic plate counts (APC), lactic acid bacteria (LAB) counts, titratable acidity (TA) and pH. Additionally, acetic acid, lactic acid, sugars (glucose, fructose, sucrose), and ethanol levels were determined using high-performance liquid chromatography (HPLC). These measurements were taken on days 1, 3, 5, 7 and 14 after inoculation. The presence of pathogens (*Vibrio, Salmonella, Staphylococcus aureus, Listeria*) was evaluated prior to inoculation and seven days after inoculation. The pH, titratable acidity (TA), instrumental texture (shear force), color, antioxidant capacity, APC, LAB counts, coliforms, yeasts, and molds were measured periodically for 60 days post inoculation.

2.2. Seaweed sauerkraut

2.2.1. Processing

Seaweed sauerkraut, consisting of farmed seaweed and cabbage, was made using sugar kelp (SK) and winged kelp (WK) freshly harvested in June 2017 from Spring Tide Seaweed (Sorrento, Maine). Harvested seaweed was delivered to the University of Maine in coolers on ice. To avoid direct contact of seaweed and ice, seaweed was placed in plastic bags. All seaweed was processed the same day it was harvested. Processing consisted of rinsing seaweed with tap water and checking for quality defects, such as epiphytes and hydroids. The poor-quality seaweeds were discarded.

Whole kelp fronds were shredded using a RobotCoupe® continuous feed food processor with a 28064 1/8" slicing disc (CL 50 Series E, Jackson, MS). SK was passed twice through the food processor, while WK was passed once through the food processor so that all materials were similar in width (~2cm). Once the kelp was shredded, it was weighed in batches according to each treatment. The 25%, 50% and 75% treatments had 1000, 2000, and 3000 g of kelp, respectively. The weighed batches were placed into two gallon Ziploc bags and put in the walk-in cooler (3 ºC) overnight.

Whole green cabbage was purchased at a local grocery store. Cabbage heads were rinsed with tap water. The outer two leaves from each cabbage head were removed and the bottom white stem was chopped off. The cabbages were quartered by hand with a sharp knife and passed through the RobotCoupe® (CL 50 Series E, Jackson, MS) once. Shredded cabbage was pre-weighed according to treatment, and stored using the same method as for the seaweed.

2.2.2. Preparation

Shredded cabbage and shredded seaweed were placed into a commercial stainless steel mixing bowl according to each treatment, for a total mass of four kg per batch. Then, 80 g (2% wt/wt) of canning and pickling salt (Morton, Manistee, MI) were added to each of the 18 batches. The salt was spread on the surface of the seaweed and cabbage mixture, and after five minutes the seaweed, cabbage, and salt were hand mixed (using gloves for sanitary purposes) vigorously for three minutes. The mixture sat for one minute and then it was mixed again by hand for one minute. This process was done to extract brine from the seaweed and cabbage mixture. Next, the contents of the bowl were packed into a one gallon glass fermentation jar (Kombucha Brooklyn, Kingston, NY) that contained a plastic lid and airlock. After the fermentation jar had been packed halfway, the jar was aseptically inoculated, using an autoclaved pipette tip and gloves, with *Lactobacillus plantarum* (10^6 CFU/g) and *Leuconostoc mesenteroides (*10^1 CFU/g). A total of 10 g of the seaweed and cabbage mixture was removed from each batch to perform microbial testing pre-and post-inoculation.

The fermentation jar was packed with the seaweed and sauerkraut mixture, with approximately two inches of headspace filled with brine above the solid materials. The jar was sealed with the airlock lid, which was filled halfway with water and monitored to ensure this level was maintained. Treatment jars were labeled and coded according to the seaweed percentage and seaweed species in the product. For example, a treatment with 75% sugar kelp and 25% cabbage was coded: SK 75. Each of the six treatments was prepared in triplicate batches (A, B, C), for a total of 18 fermentation jars. The seaweed and cabbage mixtures were fermented in a lab at ambient temperature (21-22° C) until a pH of < 4.6 was achieved to prevent growth of *Clostridium botulinum*. Subsequently, the fermentation jars containing the product

were placed in refrigerated storage (3º C) for 60 days post inoculation. Samples (brine and/or vegetable material, depending on analyses) were aseptically taken from each fermentation jar periodically to perform microbiological, pH, TA, instrumental texture, color, antioxidant capacity, and HPLC analyses.

2.3. Inoculation

2.3.1. *Lactobacillus plantarum*

Lactobacillus plantarum (ATCC 8014) was obtained in Kwik-Stick™ form from Microbiologics (St. Cloud, MN) and it was stored in the refrigerator at 2°-5°C prior to use. The *L. plantarum* was streaked onto a Lactobacilli MRS agar plate (Alpha Biosciences, Baltimore, MD) at room temperature (21-22º C) and placed into a 35°C incubator for 48 hours. White colonies formed on the MRS plate after 48 hours.

To calculate the amount of bacteria needed to inoculate the seaweed/cabbage mixture to the desired 10^6 CFU/g, the population count was determined after 24 hours of growth. To do this, one single colony was aseptically isolated from an incubated MRS agar plate and was placed into a test tube with nine mL of room temperature Lactobacilli MRS broth (Alpha Biosciences, Baltimore, MD). The *L. plantarum* tube was incubated for 24 hours and 1:10 serial dilutions were performed in 0.1% peptone (BD, Sparks, MD). Each dilution was plated, using aseptic technique, onto new MRS plates and incubated for 48 hours. The colony forming units (CFU) of *L. plantarum* per mL of sample broth were determined by the plate count number and multiplied by the dilution factor. This process was repeated ten times to calculate an average population count. The average growth after 24 hours was ~4 $X10^9$ (~log 9) and this value was used to estimate the volume (mL) of inoculate needed for each fermentation jar.

2.3.2. *Leuconostoc mesenteroides*

Leuconostoc mesenteroides was isolated from Choozit Cheese Cultures (Danisco, Paris, France). Approximately 0.5 g of this powder was directly placed in nine mL of MRS broth and incubated for 24 hours at 40º C. Then, 0.1 mL was plated onto MRS plates and incubated upside down for 48 hours at 40º C. A single colony was used from the MRS plate and the same inoculation process was used as for *L. plantarum*.

To calculate the amount of *L. mesenteroides* needed to inoculate the seaweed/cabbage mixture to the desired 10^1 CFU/g, it was assumed that the population growth dynamics in 24 hours were similar to *L. plantarum*. Jeong et al. (2017) studied the growth of *L. mesenteroides* and found that the population was 9.85 x 10^8 (~9.60 log CFU/g) after 24 hours, similar to *L. plantarum*.

2.4. Microbiological analysis

Cabbage, winged kelp, and sugar kelp were each weighed (10 g) into sterile stomacher bags and combined with 90 mL of sterile 0.1% peptone (BD, Sparks, MD). Samples were automatically homogenized (Interscience BagMixer®, Woburn, MA) for two minutes. Appropriate serial dilutions were carried out in sterile 0.1% peptone. To conduct microbial analysis of the sauerkraut treatments, one mL of brine was removed from the treatments and serially diluted with 0.1% peptone.

2.4.1. Aerobic plate counts (APC)

Aerobic plate counts (APC) using tryptic soy agar (TSA) (Alpha Biosciences, Baltimore, MD) plates were measured using aseptic techniques on days 0, 1, 3, 7, 14, 28, and 60. The TSA plates were prepared according to manufacturer's instructions (Alpha Biosciences, Baltimore, Maryland). One mL of brine was sampled from each treatment replicate. Serial dilutions in 0.1%

peptone (BD, Sparks, Maryland) were plated in duplicate. TSA plates were incubated upside down for 48 hours at 37 ºC and colonies were counted.

2.4.2. Lactic acid bacteria counts (LAB)

The lactic acid bacteria populations were counted using Lactobacilli MRS agar (Alpha Biosciences, Baltimore, MD). One mL of sample brine was aseptically removed from each treatment replicate on days 0, 1, 3, 7, 14, 28, and 60. Serial dilutions in 0.1% peptone (BD, Sparks, Maryland) were prepared based on the total plate counts and plated in duplicate, accordingly. MRS plates were incubated upside down for 48 hours at 40º C and colonies were counted. The LAB plate counts were based on colony counts of 25-300 per plate. The plate count number was multiplied by the dilution factor and LAB was calculated as colony forming units (CFU/g).

2.4.3. Coliform counts

Coliform counts were obtained using the three tube Most Probable Number (MPN) method (FDA, 2010; Hardy Diagnostics, 2018). Nine separate tubes of ten mL of lactose broth (Acumedia, Lansing, MI) were inoculated with 3 x 1mL, 3 x 0.1 mL, 3 x 10 μL brine and incubated at 37 ºC for 24 hours. After 24 hours, the test tubes were examined for turbidity and bubbles. The scoring of the most probable number was determined by comparing the number of positive test tubes to the table from the US Food and Drug Administration (FDA) Bacterial Analytical Manual (FDA, 2018). Presumptive positives were confirmed using brilliant green bile lactose broth (BGLB) (BD Biosciences, Maryland). Each of the BGLB tubes was inoculated using a loophole from the positive MPN tubes and they were incubated at 37º C for 24 hours and checked for bubbles.

2.4.4. Fungi

Acidified potato dextrose agar (APDA) plates were made using potato dextrose agar (Alpha Biosciences, Baltimore, MD) and 10% tartaric acid (pH 3.5) to measure yeast and mold growth according to manufacturer's instructions from Hardy Diagnostics (2018). A one mL aliquot of sample brine was taken out of each treatment replicate. Serial dilutions prepared in nine mL peptone broth were plated. APDA plates were incubated at room temperature (21-22º C) for 5+ days and the number of yeast and molds were counted after 7 days.

2.4.5. Pathogens

Pathogens were checked in the raw cabbage and kelps used to make the seaweed sauerkraut and in each of the treatments before inoculation and at the end of the first week of fermentation. Pathogens tested were those reasonably likely to be present in a typical processing facility. *Vibrio* spp. was also inspected due to its ubiquitous presence in the marine environment.

Methods from the Bacteriological Analytical Manual for *Staphylococcus aureus, Salmonella*, *Listeria monocytogenes,* and *Vibrio* (FDA, 2018) were modified to enumerate the pathogens accordingly. To identify each pathogen, 25 g of each of the test materials were placed aseptically into a 225ml pathogen specific broth, placed in a stomacher bag for two minutes, incubated in stomacher bags for 24-48h, then streaked and spread plated (0.1mL) in duplicate onto pathogen-specific plates (Table 1). The plates were checked for growth to determine the presence of pathogens.

Table 1. Media, incubation time, and temperatures used to detect different pathogens.

Pathogen	Media	Incubation Time and Temperature
Staphylococcus aureus	Tryptic soy broth (Acumedia, Lansing, MI) with 10% salt (NaCl) and 1% sodium pyruvate (TSBSP) incubated at 35º C for 24 h.	Baird-Parker (Alpha Biosciences, Baltimore, MD) plates incubated at 35º C for 48 h.
***Salmonella* spp.**	Lactose broth (Acumedia, Lansing, MI) incubated at 35 ºC for 24 h.	Xylose lysine deoxycholate agar (XLD) (Alpha Biosciences, Baltimore, MD) incubated at 35º C for 48 h. The plates were checked for colonies with black centers.
Listeria monocytogenes	Listeria enrichment broth (LEB) (Alpha Biosciences, Baltimore, MD) incubated at 28º C for 24 h.	Modified oxford agar base (MOX) (Alpha Biosciences, Baltimore, MD) plates incubated at 28º C for 48 h.
***Vibrio* spp.**	Alkaline peptone water (pH 8.6) (Alpha Biosciences, Baltimore, MD) incubated at 28º C for 24 h.	Thiosulfate-citrate-bile salts-sucrose agar (TCBS) (Alpha Biosciences, Baltimore, MD) plates incubated at 28º C for 48 h.

2.5. pH

The pH measurements of each treatment batch were taken using a 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 seaweed sauerkraut were aseptically removed from the fermentation jars. The flat probe was placed directly on the seaweed sauerkraut until a consistent reading was obtained. Measurements were taken in duplicate per fermentation jar and pH values were averaged. pH measurements were taken on days 1, 3, 5, 7, 14, 28 and 60 post inoculation.

2.6. Titratable acidity

A modified titratable acidity (TA) procedure (UC Davis, 2018) was used as an approximation of total acidity in the treatments on days 1, 3, 5, 7, 14, 28, and 60. Seaweed sauerkraut (10 g) from each treatment was aseptically removed from the fermentation jars and

the weight was recorded (0.1g). The sample was placed into a beaker with 100 mL of distilled water and a magnetic stir bar. A pH meter, 0.1 N sodium hydroxide (NaOH) (Fisher Scientific, Waltham, MA), and a magnetic stir plate were set-up to perform the titration. The sodium hydroxide was slowly titrated into the beaker until the standard pH of 8.2 was achieved. The total volume (mL) of titrant added was recorded.

The calculations were based on the acidity of lactic acid, where the volume of sodium hydroxide that was added was converted to moles of lactic acid and multiplied by the equivalent milligrams of lactic acid and divided by the original weight of the sample (g). TA was measured as a percentage value according to the following formula:

$$\frac{\text{Normality of NaOH (mol/L)} * \text{Volume of NaOH (mL)} * \text{Equivalent weight of lactic acid (g/mol)}}{\text{Sample mass(g)} * 1000} * 100 = \% \text{ TA}$$

2.7. Texture analyses

A Kramer shear force method was used to evaluate the texture of the seaweed sauerkraut, using methods modified from Johanningsmeier et. al (2007). The texture analyzer (TA-XTi2, Texture Technologies Inc., Scarsdale, NY) was calibrated using a 5,000 g load cell from the same company before each use. Twenty-five g of seaweed sauerkraut were loaded in a single layer on the base of the Kramer shear cell (TAXTi2, Texture Technologies Inc., Scarsdale, NY), with a total of five flat blades. The pre-test and post-test speed was set to 2 mm/sec with a distance of 50 mm (Johanningsmeier et. al 2007). 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). The test was repeated ten times for every treatment replicate, and force values were averaged.

2.8. Colorimetric analyses

Colorimetic analysis was performed using a LabScan XE Colorimeter (HunterLab) to determine L*, a*, and b* values. Before each use, the colorimeter was warmed for 30 minutes and was standardized using black and white ceramic standard plates. The area view was set to 1.75 inches and the port size was set to 5. Colorimeter sample cups (353002, Corning, Durham, NC) were filled to the top with seaweed sauerkraut. A total of three readings were taken and averaged by the software by rotating the colorimeter cup slightly clockwise after the initial reading. Ten subsamples were measured for each treatment replicate and averaged.

2.9. Antioxidant analysis

2.9.1. Sample preparation

Approximately 50 g of seaweed sauerkraut from each treatment replicate were taken at days 21, 42, and 60 of the experiment. Antioxidant analysis was performed at these times because they represented likely time points when typical consumers would receive this product. The samples were placed in plastic bags (Nasco Whirlpack, Fort Atkinson, WI), placed in the blast freezer (-30º C) (Southeast Cooler, Lithia Springs, GA) for 1 hour, and then stored at -80 ºC for three weeks. Samples were later processed using a freeze drier (35 EL, VirTis Co. Inc. Gardiner, NY) for 72 hours (24 hours for each cycle) until the sample weights no longer fluctuated. The dried samples were crushed and ground by using a mortar and pestle. The ground samples were stored in plastic bags (Nasco Whirlpack, Fort Atkinson, WI) in the freezer (-80 ºC).

The ground samples were thawed and weighed to 2 g (± 0.005 g) and 20 mL of 60% methanol (Fisher Scientific, Waltham, MA) was added to the samples in an Erlenmeyer flask, 10 mL, at a time. The samples were placed on a shaker for 24 hours at 210 rpm (13687700 Multi-

Platform Shaker, Fisher Scientific, Waltham, MA) with parafilm to cover each Erlenmeyer flask and to minimize sample evaporation. Next, the samples were centrifuged for 10 minutes at 2100 xg (Beckman Avanti J-25, Brea, CA). The supernatant was collected and 10 mL of 60% methanol was added to the centrifuge tube and vortexed for 30 seconds to mix the solution. The sample was placed back on the shaker for ten minutes and then centrifuged again. This process was repeated two more times and the supernatant volumes were combined. After this process, distilled water was added to the supernatant to bring the total volume to 50 mL. The solution was vortexed for 30 seconds. This extract was used for all antioxidant analysis and it was kept no longer than 48 hours at -20º C.

2.9.2. Total phenolic content (TPC)

The Folin-Ciocalteu method was used to measure the total phenolic content in the sauerkraut samples (Matanjun et al. 2008, Rajauria et al. 2010). The Folin-Ciocalteu reagent (Sigma Aldrich, St. Louis, MO) was diluted (1:10) in distilled water prior to use. The diluted Folin (1.5 mL) was added to 200 µL of sample extract, then the solution was vortexed for ten seconds. After a five-minute incubation period, 1.5 mL of 6% sodium bicarbonate solution was added. The samples were then placed in the dark for 1 hour to incubate.

Stocks of varying amounts of gallic acid (TCI Chemicals, Portland, OR) (0-200ug/mL in 40% methanol) were used as a standard. Samples were run in duplicate and the values were averaged per treatment replicate. The absorbance was measured at 725 nm using a spectrophotometer (Beckman Du 530, Brea, CA) and the blank for this protocol was 40% methanol. Total phenolic content was expressed as mg gallic acid equivalents per g of freeze-dried sample.

2.9.3. Ferric reducing antioxidant power (FRAP) assay

The Benzie and Strain (1996) method was used with some modifications to perform FRAP analysis. Four different solutions (A, B, C, D) were made to perform the FRAP analysis. Solution A was a 300 mM acetate buffer (pH 3.6) that was made by adding 3.1 g sodium acetate trihydrate (Fisher Scientific, Waltham, MA) to 16 mL glacial acetic acid (Fisher Scientific Waltham, MA). The solution was brought up to 1000 mL (1L) with distilled water. Solution B was 10 mM 2,4,6-tripyridyl-s-triazine (TPTZ, Acros, Fair Lawn, NJ) in 40 mM hydrochloric acid (HCl, Fisher Scientific Waltham, MA). Solution C was 20 mM $FeCl_3 6H_20$ (Acros, Fair Lawn, NJ). Solution D (FRAP reagent) was a mixture of Solution A, Solution B, and Solution C (10:1:1) that was stirred until well mixed. Solution D was warmed to 37º C in a water bath prior to use. A standard curve was comprised of varying concentrations (50-500 μM) of $FeSO_4 7H_2O$ in deionized water (Spectrum Chemicals, New Brunswick, NJ). To conduct this assay, one mL of sample extract or standard was placed in a cuvette and 3 mL of warmed FRAP reagent was added. After 4 minutes, the absorbance was measured at 593 nm against a deionized water blank. An internal control of 250 μM Trolox (Acros, Fair Lawn, NJ) in 40% MeOH (Fisher Scientific, Waltham, MA) was used. Each sample extract was analyzed in duplicate and results were expressed in μmol ferrous sulfate equivalents (FSE) per gram of freeze-dried sample.

2.9.4. α, α-diphenyl-β-picrylhydrazyl (DPPH) assay

DPPH was performed based on the method from Blois (1958) with modifications. A 0.2 mM DPPH (Sigma Aldrich, St. Louis, MO) solution was prepared in 100% ethanol. To conduct the assay, varying amounts (0.5, 1.0, 1.5, 2.0 mL) of the sample extracts were each pipetted into test tubes, total volumes were brought to 2 mL using 40% methanol and vortexed for 10 seconds.

Next, 2 mL of DPPH solution was added to the test tubes which were then vortexed for 10 seconds. The test tubes were incubated in the dark for 30 minutes.

After a 30-minute incubation in the dark, the absorbance was measured at 517 nm against the blank of 100% ethanol. Duplicate sample extracts were prepared the same way to make sample blanks that would account for the purple color of DPPH. Therefore, 2 mL of ethanol was added to the 2 mL of seaweed extracts instead of DPPH solution. The make the control, 2 mL of either DPPH solution or ethanol was added to 2mL of 40% methanol. In addition, Trolox standards were also made in duplicate for this procedure to serve as a positive control. To make the Trolox standards, varying volumes (0.25, 0.50, 1.0 1.5, 2.0 mL) of Trolox were added to test tubes and brought up to 2 mL with 40% methanol and 2 mL of ethanol was added to half of the test tubes and 2 mL of DPPH solution was added to the other half. The following formula was used to calculate % inhibition:

$$\% \text{ DPPH inhibition} = \text{Control Abs} - \left(\frac{\text{Sample Abs} - \text{Sample Blank Abs}}{\text{Control Abs (DPPH and 40\% MeOH)}}\right) \times 100$$

The calculated % DPPH inhibition values were plotted against the concentrations (mg/mL) of sample and the effective concentration (mg/mL) of sample required to inhibit 50% of the DPPH radical (EC_{50}) was calculated using the slope and constant of the plotted line.

2.10. High performance liquid chromatography (HPLC)

HPLC analysis was performed on brine samples to quantify acetic acid, lactic acid, glucose, sucrose, fructose, and ethanol concentrations. Brine samples (1 mL) were collected on days 1, 3, 5, 7, and 14 of the study and stored at -20° C. Samples were thawed in the refrigerator, diluted (1:3) in mobile phase (0.01M H_2SO_4), then passed through 0.45 μm filters (Advanced Microdevices MDI Membrane Technologies, Ambala Cantt, India) using a 5-mL plastic syringe.

Filtered samples were placed into 2 mL vials and loaded in the HPLC (Agilent Technologies, Santa Clara, CA). The HPLC system consisted of an 1100 series pump, degasser and autosampler, and a 1200 series refractive index detector (RID). The 0.05 M H_2SO_4 mobile phase temperature was set at 50° C and the injection volume was 20.0 µL. The column (Agilent Hi-Plex H) flow was 0.6 mL/min. Standards of sucrose, glucose, and fructose ranging from 0.0025-0.03 mg/mL were prepared to identify and quantify sugars in sample brines. Standards ranged from 0.0001-0.01 mg/mL for acetic acid and from 0.0003-0.01 mg/mL for lactic acid. For ethanol, standards ranged from 1-3 mg/mL.

2.11. Statistical analysis

Data were analyzed using IBM SPSS Statistics 24. Outliers were removed using a 3 X Interquartile range (IQR). Multi-way analysis of variance (ANOVA) was used to assess the main effects of seaweed species (2 levels), seaweed concentration (3 levels), and time (variable levels, depending on analysis) on dependent variables (Laerd Statistics, 2018). Tukey's Honest Significant Difference (HSD) test was selected for post-hoc analyses. A significance value of $p<0.05$ was chosen for all statistical analyses. A Spearman Correlation was used to assess the linear relationships between select dependent variables.

2.12. Sensory testing

Consumer acceptability testing occurred at the University of Maine Sensory Evaluation Center (SEC) in Hitchner Hall on Tuesday, March 27th 2018 between the hours of 11:00 am and 5:00 pm. Approximately one hundred participants who enjoy consuming sauerkraut were recruited via word of mouth, e-mail, and posted flyers to rate the acceptability of the seaweed sauerkraut (Appendix A). Prior to testing, institutional review board (IRB) approval was obtained. An informed consent was provided before participation and those allergic to seafood

and those who do not enjoy consuming seaweed or sauerkraut were asked to not participate in the sensory evaluation (Appendix B). The consumers received a three dollar incentive for their participation.

2.12.1. Seaweed sauerkraut preparation for sensory testing

All samples were processed using fresh seaweed collected on March 1, 2018 from Spring Tide Seaweed (Sorrento, Maine). The seaweed sauerkraut was prepared using the same methods as described previously. A total of 3000 g of shredded seaweed and cabbage were weighed, mixed with 2% canning and pickling salt, inoculated with *L. plantarum* (10^6 cfu/g) and *Leuconostoc mesenteroides (*10^1 cfu/g), packed into four fermentation vessels, and allowed to ferment at room temperature. To guarantee the safety of these products, the presence of pathogens (*E. coli, Vibrio*) was checked and the end pH in the samples was verified as < 4.0. This pH was below the minimum pH of 4.6 needed to control the growth of *Clostridium botulinum*. Once the desired pH was established, the samples were placed in the walk-in cooler (3º C) for two weeks until sensory evaluation to simulate when a typical consumer might receive the commercial product after transport and stocking.

2.12.2. Sample testing

Consumers tested four seaweed sauerkraut samples: two sugar kelp and two-winged kelp formulations made with 25 and 50 percent seaweed concentrations. The samples were served chilled and panelists were asked to evaluate the texture, color, flavor, aroma, and overall acceptability of the products using a 9-point hedonic scale (1 = dislike extremely, 5 = neither like nor dislike, 9 = like extremely) (Meilgaard et al. 2016) (Appendix C). Just about right scales (1=Not nearly salty/sour enough, 3=Just about right, 5=Much too salty/sour) were used to evaluate sourness and saltiness. Prior to testing the samples, panelists were asked general demographic questions and how often they consumed seaweed, sauerkraut, and probiotics.

Lastly, the participants were asked to add any additional comments about the samples they tested.

The test process was executed using SIMS 2000 software (SIMS Sensory Quality Panel Software Cloud Systems, Sensory Computer Systems, Berkeley Heights, NJ) and the acceptability data were assessed by one-way Analysis of Variance (ANOVA) and Tukey's post-hoc test to determine significant ($p < 0.05$) differences among the four samples.

During testing, the environmental conditions were kept clean and well-lit to control variables and biases. Distractions were kept to a minimum and differences were minimized amongst samples by filling each ramekin half full (~25 g) of seaweed sauerkraut. Samples were placed into two-ounce clear plastic containers that had computer printed labels attached at the same location. All samples were kept at a similar temperature by holding them in the refrigerator until serving. The presentation of samples was balanced and randomized according to the SIMs software. The participants were asked to evaluate the four coded samples from left to right and to take a sip of water in between samples.

3. RESULTS AND DISCUSSION

3.1. Overview

The results indicate that kelps can be fermented successfully and that seaweed species and incorporation level affect certain characteristics of the fermented products. Visually, the winged kelp (WK) sauerkraut was slightly darker green and the fermentation vessels had more brine. Seaweed and cabbage were clumped together in the SK treatments and were somewhat slimy to the touch. Some SK treatments also had a slight red color, possibly from yeast contamination, at the upper surface of the sauerkraut and this have may been due to air exposure from having less brine in the treatments or from repeated sampling. Each fermentation jar was filled to the top with brine to normalize the amount of brine across treatments and to avoid exposure to air, however some samples bubbled over during fermentation resulting in some loss of brine. Furthermore, fermentation jars had to be checked frequently to make sure that samples were covered in brine. To avoid this in future studies, fermentation weights could be used to help keep the samples more packed and submersed in brine. The bubble production in the air lock showed that all treatments produced carbon dioxide, however less bubbling occurred in the high SK concentration treatments. No spoilage off odors were noted during the experiment and overall, these products remained stable throughout the refrigerated storage study.

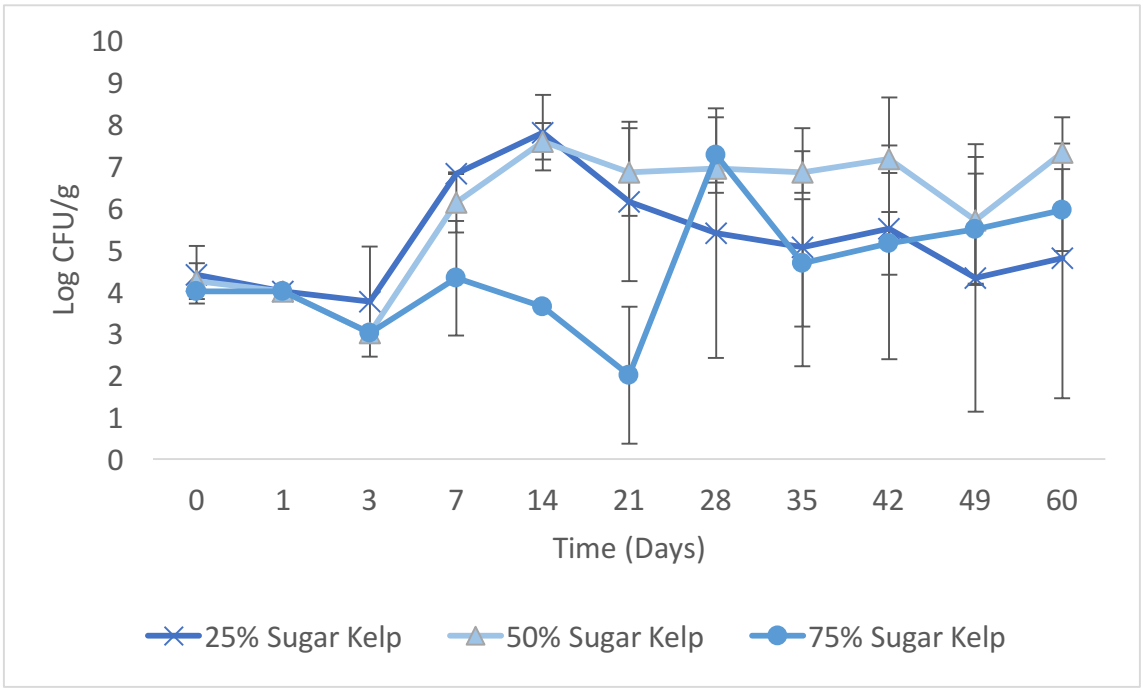
3.2. Microbiology

3.2.1. Lactic acid bacteria counts

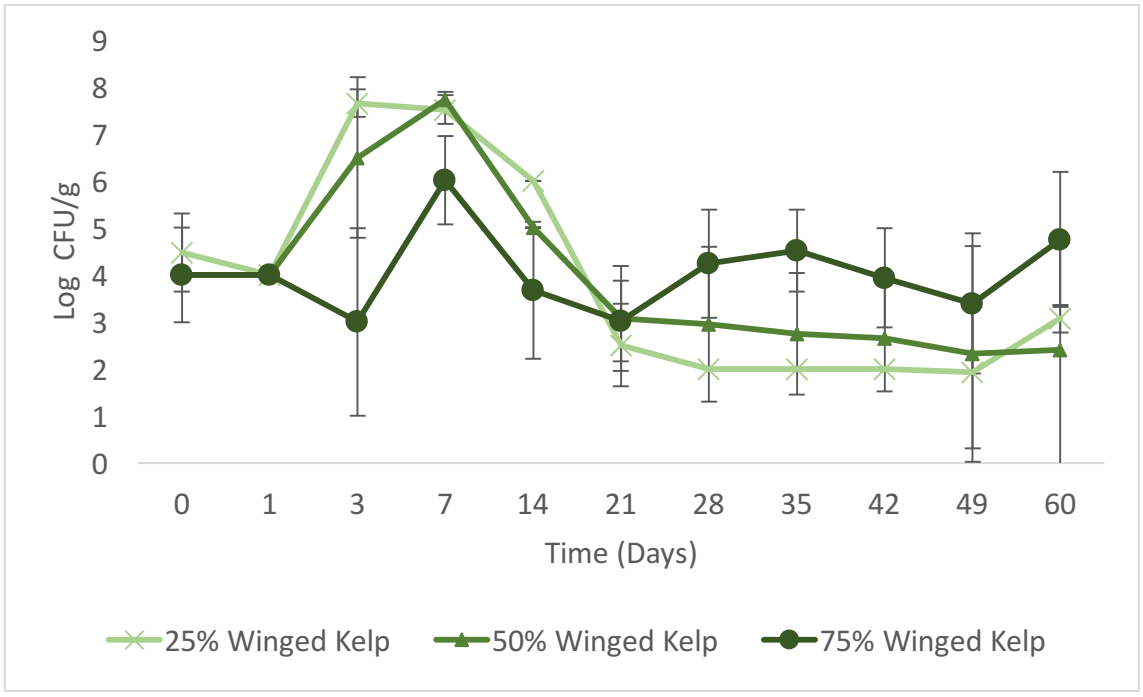
Each treatment was inoculated with *Lactobacillus plantarum (*target 10^6 CFU/g*)* and *Leuconostoc mesenteroides* (target 10^1 CFU/g*)* at the beginning of the study (Day 0). The lactic acid bacteria count immediately post inoculation was less than 4 log CFU/g in all treatments. This was lower than expected, possibly because the microbial sampling for this time point was taken shortly after inoculation. The average lactic acid bacteria count in the seaweed sauerkraut

treatments ranged from 3.00- 7.79 log CFU/g over time as the counts increased throughout the study (Figure 5). However, all treatments reached an average lactic acid bacteria count of 10^6 CFU/g or higher during the study. The increase in total lactic acid bacteria populations over time was expected due to fermentation and the use of starter cultures. Furthermore, the high ($\approx 10^6$) lactic acid bacteria populations found in the treatments show the potential of this product as a probiotic. Beganovic et al. (2011) defined probiotic products as containing at least 10^6 live probiotic colonies per gram and the added *Lactobacillus plantarum* and *Leuconostoc mesenteroides* are both considered probiotic strains (Beganovic et al. 2011). Additionally, the maximum lactic acid bacteria populations found in this study are comparable to an inoculated 2.5% NaCl sauerkraut study by Beganovic et al. (2011), where counts ranged from 5.88-7.27 log CFU/mL.

Figure 5. Mean lactic acid bacteria populations (log CFU/g) in seaweed sauerkraut over time, a) sugar kelp and b) winged kelp.



a)



b)

Values are means ± S.D. (n=3).

There was a significant ($p=0.03$) interaction between seaweed species, concentration, and time with regard to LAB population. Seaweed species had a significant effect on LAB counts in the sauerkraut, as SK had a significantly ($p<0.01$) higher (5.42 log CFU/g) average lactic acid bacteria population overall compared to WK (3.97 log CFU/g). The higher overall LAB counts in the SK treatments is because their LAB counts remained steady over time. Additionally, there was a significant interaction between time and treatment ($p<0.01$). After day 7 of the study, mean LAB counts across SK treatments varied by only 1 log CFU/g. In contrast, the LAB counts over time in WK dropped similarly to an inoculated sauerkraut fermentation study on red cabbage (Hunaefi et al. 2013). The authors reported maximum LAB counts on day 7 and after the maximum was reached, the population decreased slowly, a trend that was also found in the WK treatments. Despite the higher average LAB counts in SK, WK reached 6% higher maximum LAB counts during the study. For WK, the maximum mean LAB counts occurred on day 7 (7.08 log CFU/g), while for SK, the maximum mean LAB count was on day 60 (6.65 log CFU/g). Overall, the highest mean lactic acid bacteria count was 6.42 log CFU/g on day 7.

The difference in LAB counts among seaweed species is interesting because both species are brown seaweeds. Brown seaweeds are rich in carbohydrates including laminarin, mannitol, and structural carbohydrates such as alginate and cellulose (Schiener et al. 2015). Their carbohydrate contents vary seasonally, as seaweed have more storage carbohydrates during the fall because they are an energy source in the colder months (Schiener et al. 2015). Carbohydrates are necessary for LAB growth and ideal carbohydrates used in seaweed fermentation are the glucose polymer laminaran and sugar alcohol mannitol (Sandbakken et al. 2018). Therefore, the changing carbohydrate composition during seasons could account for the differences in LAB counts among species. A study compared the seasonal differences in the

composition of WK and SK and found that WK had maximum carbohydrate content during early June and July (Schiener et al. 2015). However, SK carbohydrate maximums were found later in fall (Schiener et al. 2015). The seaweed was collected in June for this study. Therefore, high WK LAB counts found in the first week of this study could be from the availability of carbohydrates in the WK and could account for the delayed fermentation in SK. Furthermore, seasonality also influences the minerals and vitamins found in seaweed species and changes the micronutrients available for microbial growth in fermentation and this could account for LAB count variability among species (Schiener et al. 2015).

There was a significant interaction between seaweed concentration and seaweed species ($p=0.01$) and seaweed concentration and time ($p<0.01$). Varying amounts of SK/cabbage influenced lactic acid bacteria growth, with significantly ($p<0.01$) lower overall LAB counts found in the 75% SK treatment. However, in the WK, the 25%, 50%, and 75% treatments were not significantly different in lactic acid bacteria populations (Table 2). Lactic acid bacteria are naturally found on cabbage and an average of 3.71 log CFU/g was present on the raw cabbage. No lactic acid bacteria were detected in raw winged kelp and sugar kelp starting material, based on a 25-300 colonies per plate detection limit. Despite the higher starting lactic acid bacteria populations in the cabbage compared to kelps, the treatments with more cabbage did not have significantly higher mean LAB populations, likely from inoculating ~100 times higher than the natural LAB population found on cabbage. However, this could also suggest that seaweed has similar nutrients to cabbage that can sustain lactic acid bacteria populations. As mentioned previously, cabbage and seaweed are rich sources of carbohydrates. There are also important

micronutrients found in cabbage and seaweed, like zinc, that help support microbial growth during fermentation (Wu et al. 2008; Schiener et al. 2015).

Table 2. Lactic acid bacteria populations (log CFU/g) of seaweed sauerkraut over time.

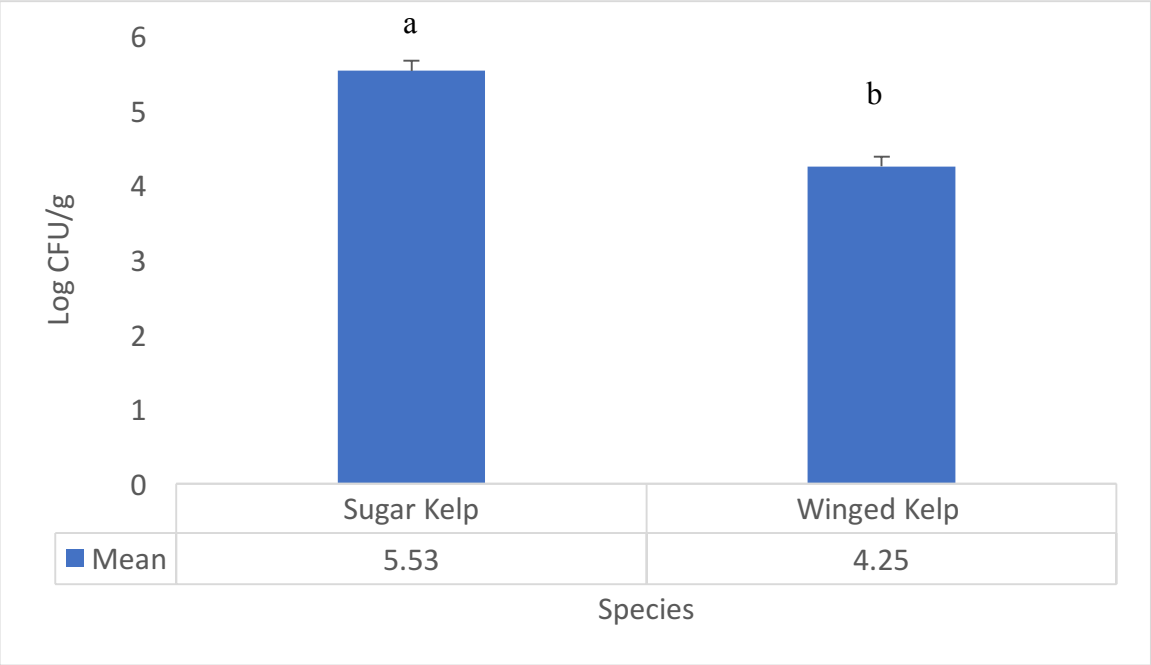
Species		**Sugar Kelp**			**Winged Kelp**		
Concentration (% seaweed)		25	50	75	25	50	75
Days	**0***	4.40 ± 0.69	4.24 ± 0.42	4.00 ± 0.00	4.48 ± 0.83	4.00 ± 0.00	4.00 ± 0.00
	1*	4.00 ± 0.00	4.00 ± 0.00	4.00 ± 0.00	4.00 ± 0.00	4.00 ± 0.00	4.00 ± 0.00
	3	3.76 ± 1.31	3.00 ± 0.00	3.00 ± 0.00	7.65 ± 0.29	6.49 ± 1.70	3.00 ± 0.00
	7	6.81 ± 0.00	6.12 ± 0.71	4.31 ± 2.88	7.51 ± 0.31	7.72 ± 0.16	6.01 ± 2.13
	14	7.79 ± 0.90	7.58 ± 0.44	3.63 ± 1.09	6.00 ± 0.00	5.00 ± 0.0	3.67 ± 0.58
	21	6.14 ± 1.90	6.84 ± 1.04	2.00 ± 0.00	2.51 ± 0.88	3.07 ± 1.11	3.02 ± 1.76
	28	5.39 ± 2.98	6.93 ± 0.34	7.25 ± 0.98	2.00 ± 0.00	2.95 ± 1.64	4.24 ± 1.76
	35	5.06 ± 2.84	6.84 ± 0.50	4.67 ± 1.60	2.00 ± 0.00	2.74 ± 1.29	4.52 ± 1.10
	42	5.50 ± 3.12	7.15 ± 0.33	5.15 ± 1.09	2.00 ± 0.00	2.66 ± 1.14	3.94 ± 1.75
	49	4.32 ± 3.18	5.69 ± 1.51	5.47 ± 1.91	1.93 ± 1.61	2.32 ± 2.29	3.39 ±2.08
	60	4.80 ± 3.35	7.32 ± 0.21	5.93 ± 0.99	3.07 ± 0.29	2.40 ± 2.43	4.75 ± 1.58

Values are means ± S.D. *Day 0 and 1 are estimates.

3.2.2. Aerobic plate counts

There was a significant interaction found among seaweed species and seaweed concentration ($p<0.01$), seaweed species and time ($p<0.01$), and seaweed concentration and time ($p<0.01$). The aerobic plate counts were 23% higher overall in the SK treatments (5.53 log CFU/g) compared to the WK treatments (4.25 log CFU/g), meaning that there was a significantly higher total bacteria population in the SK treatments (Figure 6). The WK aerobic plate count remained consistent over time among different seaweed concentrations, with a mean ranging from 4.23-4.28 log CFU/g (Table 3). All WK concentrations had significantly lower average aerobic plate count throughout the study when compared to SK treatments. In contrast, the addition of SK affected the aerobic plate count, as the highest average aerobic plate count was in the 50% treatment (6.33 log CFU/g), while the lowest average aerobic plate count was in the 75% treatment (4.69 log CFU/g). These results were unusual, however, this could have been due the antimicrobial effects of seaweed (Kausalya and Narasimha 2015

Figure 6. Mean aerobic plate counts (log CFU/g) in seaweed sauerkraut by species.



Values are means ± S.E. Treatments not sharing a letter are significantly ($p<0.001$) different based on Multi-Way ANOVA (n=9).

Table 3. Aerobic plate counts (log CFU/g) of seaweed sauerkraut over time.

Species		Sugar Kelp			Winged Kelp		
Concentration (% seaweed)		25	50	75	25	50	75
Days	0	4.00 ± 0.00	4.00 ± 0.00	4.00 ± 0.00	4.00 ± 0.00	4.19 ± 0.33	4.58 ± 1.01
	1	3.50 ± 0.09	4.00 ± 0.00	4.00 ± 0.00	5.20 ± 0.08	5.43 ± 0.36	4.00 ± 0.00
	3	4.98 ± 0.09	3.62 ± 2.62	2.00 ± 0.00	6.69 ± 0.21	5.81 ± 0.40	3.15 ± 1.99
	7	7.72 ± 0.16	6.72 ± 0.66	5.40 ± 1.37	8.72 ± 0.16	6.84 ± 1.26	5.42 ± 0.94
	14	8.36 ± 0.90	5.35 ± 4.40	4.00 ± 0.00	6.00 ± 0.00	6.00 ± 0.00	4.29 ± 1.45
	21	6.54 ± 2.22	7.29 ± 0.62	2.95 ± 1.64	3.81 ± 0.26	3.57 ± 1.41	2.98 ± 0.85
	28	5.52 ± 3.08	7.07 ± 0.39	6.77 ± 0.90	3.13 ± 1.02	3.11 ± 1.92	4.42 ± 1.15
	35	5.15 ± 2.85	6.95 ± 0.51	5.83 ± 1.52	2.00 ± 0.00	2.82 ± 1.42	4.79 ± 0.87
	42	5.15 ± 2.74	7.22 ± 0.24	5.28 ± 0.74	2.00 ± 0.00	2.80 ± 1.39	4.45 ± 1.05
	49	4.16 ± 3.24	6.34 ± 0.76	5.95 ± 1.33	2.44 ± 0.77	3.30 ± 1.55	3.72 ± 1.49
	60	4.92 ± 3.45	5.06 ± 3.95	5.47 ± 0.98	2.53 ± 1.37	3.42 ± 2.47	4.75 ± 1.44

Values are mean ± S.D. (n=3).

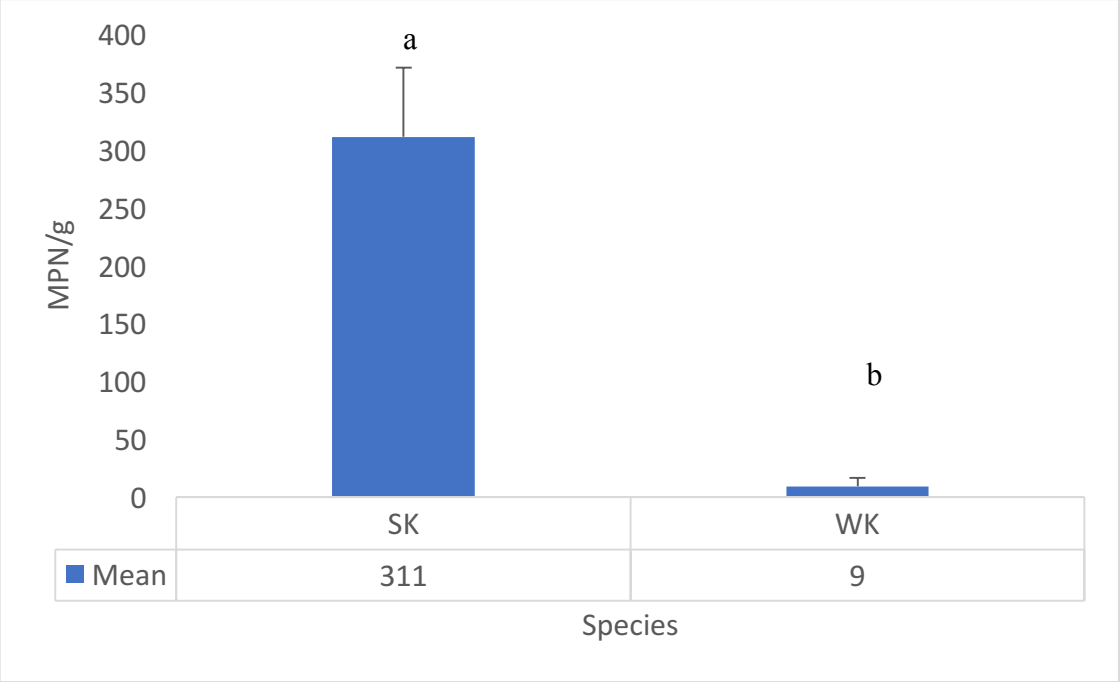
However, the mean aerobic plate count across all treatments was similar at the beginning (day 0) and the end (day 60) of the study, as the mean aerobic plate count started at 4.13 log CFU/g and ended at 4.77 log CFU/g. Throughout the study, the overall aerobic plate count ranged from 3.08-6.99 log CFU/g across all treatments over time. The aerobic plate counts were considerably lower compared to another sauerkraut study by Beganovic et al. (2011) that tested three different conventional sauerkraut formulations. One treatment had 4% salt and was a spontaneous fermentation. The other two treatments were inoculated with *L. plantarum* and *L. mesenteroides* (1:1), with one treatment having 2.5% salt and the other having 4% salt. The mean range of the aerobic plate count among all these treatments was 6.04 to 8.11 log (Beganovic et al. 2011). The addition of seaweed to the treatments could account for the lower total bacteria population because compounds in seaweed display antimicrobial activity (Perez et al. 2016). Brown seaweeds (like kelp) are rich in polysaccharides and phenolic compounds. Because of the unique structure and chemical nature of these compounds, they are known for antimicrobial, antiviral, and antifungal activity (Perez et al. 2016). Furthermore, seaweeds produce metabolites that act as defense mechanisms during environmental stress and act as natural antimicrobial agents (Perez et al. 2016).

3.2.3. Coliform counts

Coliforms are typically tested to determine water quality and to detect fecal coliforms, which are evident in water polluted with sewage or animal waste (CDC, 2015). While the number of coliforms in the seaweed sauerkraut varied randomly throughout the study, positive MPN tubes were confirmed. This indicates that fecal coliforms were present in some of the treatments. The cabbage could have contributed to the number of coliforms could that were found, as the average number of coliforms found on the raw cabbage was 47 times higher than

the average number of coliforms found on the winged kelp (15 MPN/g) and sugar kelp (14 MPN/g). Seaweed could have also contributed to these values, if so, indicating that the harvest waters and seaweed were exposed to coliform bacteria. To support this, the concentration of seaweed significantly influenced the number of coliforms that were found in the SK seaweed sauerkraut. The 75% SK had significantly higher coliform counts than the 25% SK and 50% SK treatments, at 619 $\pm$ 105.45 MPN/g, 229.6 $\pm$ 101.87 MPN/g and 85.57 $\pm$ 101.87 MPN/g, respectively. Contrastingly, there were no significant differences in coliform counts as the WK concentrations increased in the treatments. However, the WK treatment fermented sooner than SK and fermentation is reported to decrease the number of coliforms found in a product (Kimmons et al. 1999). This could explain the lower number of coliforms detected in WK sauerkraut and why coliform counts were significantly higher in the SK treatments throughout the entire study (Figure 7). Additionally, the 75% SK treatments fermented the slowest and this could also be why this treatment had the highest number of coliforms. Overall, the process of fermentation did not decrease the number of coliforms, as much as anticipated. Kimmons et al. (1999) defined product contamination as greater than 100 CFU/mL of sample when measuring coliforms in different foods. Several seaweed sauerkraut treatments had higher numbers recorded. Overall, length of storage did not influence the number of coliforms that were found in the sauerkraut treatments.

Figure 7. Mean positive coliform count (MPN/g) in seaweed sauerkraut by species.



Values are means (MPN/g ± S.E.) Values not sharing a letter are significantly ($p<0.01$) different from each other based on Multi-Way ANOVA (n=9).

3.2.4. Fungi

The presence of mold and yeast was monitored throughout the entire study on a weekly basis. For the most part, mold and yeast were not present throughout the study. Molds were sometimes detected, but most treatments had an average of less than one mold present each week. Additionally, the presence of mold and yeast was random among the treatments in the study.

Molds were not expected in these products, as molds typically grow in aerobic conditions and the seaweed sauerkraut was sealed and covered in brine. However, yeasts are typical spoilage organisms in a fermented product. The use of the starter culture, *L. plantarum,* in this study helped to avoid yeast contamination among the treatments. Research by Prachyakij et al.

(2008) found that *L. plantarum* enabled the best yeast inhibition in fermented plant beverages when tested against 72 different LAB strains and helps support the results in this study.

3.2.5. Pathogens

Food safety is important to maintain in all products and pathogens are typically responsible for foodborne illness. Therefore, the presence of *Staphylococcus aureus, Salmonella, Listeria monocytogenes,* and *Vibrio* was checked in all treatments. One pathogen, *Vibrio,* was detected at the start of the study on the raw winged kelp material. Further testing showed that *Vibrio* was also present in the first week of the study on the sugar kelp 75% treatment, but it was not present at the end of the study. The presence of this pathogen could be because the seaweed in this study was harvested at the end May, which is towards the end of the harvesting season. *Vibrio* is a pathogen that is typically more prominent in the summer/warmer months and this could be why it was detected (CDC, 2017). However, kelp is usually harvested earlier in the spring and this could help prevent contamination by *Vibrio*. When samples were prepared for sensory evaluation using seaweed that was collected in March, no pathogens were detected. Additionally, collecting the seaweed in March resulted in much cleaner seaweed overall. There was hardly any biological fouling present, meaning that hydroids, epiphytes, and other organisms were not on the seaweed. There was a much larger incidence of biological fouling on the seaweed collected in May.

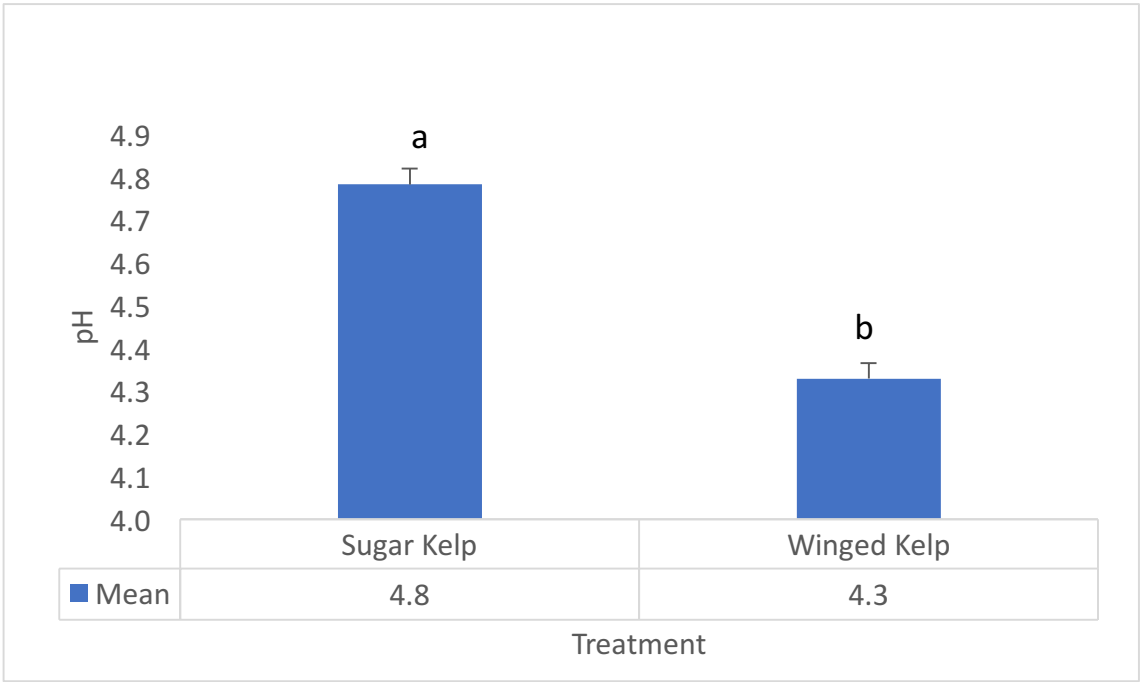
It should be reiterated that the absence of pathogens, low aerobic plate count, and low presence of mold/yeast, could be because of the important antimicrobial effects of seaweed (Kausalya and Narasimha 2015; Perez et al. 2016; Roohinejad et al. 2017). The inhibition of pathogens was tested in a study by Kausalya and Narasimha (2015). The growth inhibition of 18 different pathogens (*S. aureus*, *E. coli, Enterobacter aerogenes,* etc.) was tested in brown

seaweed extracts and it was determined that seaweed had significant antimicrobial activity based on the absence of pathogens.

3.3. pH

pH is a key indicator used to track fermentation, as acid is the main product in a lacto-fermentation. Kelp species had a significant ($p<0.01$) effect on pH values of sauerkraut treatments, with WK treatments having lower overall pH values than SK treatments (Figure 8). There was also a significant ($p=0.02$) interaction between species and concentration. In both species, the 25% treatments had overall pH values that were significantly ($p=0.002$) lower than the 75% seaweed concentrations. As the concentration of SK increased there was no significant effect on pH values. However, as the concentration of WK increased from 50% to 75% the pH increased significantly ($p<0.001$) from 4.2 to 4.5.

Figure 8. Mean pH values in seaweed sauerkraut by species.



Values are means ± S.E. Values not sharing a letter are significantly different ($p<0.001$) from each other based on Multi-Way ANOVA ($n=9$).

Based on pH, there was a significant ($p=0.02$) three-way interaction between seaweed species, percent, and time. Regarding time and species, there was a significant ($p<0.01$) interaction and WK treatments fermented quicker than the SK treatments. All WK treatments reached the goal pH of 4.6 or lower by day 3. The results are consistent with other sauerkraut fermentation studies that inoculated with *L. plantarum* and *L. mesenteroides*. Hunaefi et al. (2013) and Beganovic et al. (2011) inoculated cabbage with *L. plantarum* and found that the sauerkraut pH reached below 4.6 by day 4 and day 7, respectively. However, in the current study, SK did not reach the goal pH until day 14. SK took approximately 11 days longer to ferment compared to WK (Table 4). The delayed fermentation in the SK treatments is consistent with the lactic acid bacteria populations, as some of the highest lactic acid bacteria populations were achieved on day 28 and 60 of the study, which is when the lowest pH values were also recorded. Furthermore, pH was negatively correlated ($p<0.001$) with aerobic plate counts (-0.30) and lactic acid bacteria (-0.35), meaning that the pH decreased when the populations increased. Although the SK treatments exhibited delayed fermentation in comparison to WK, the fermentation times were quicker than in typical spontaneous sauerkraut products (Beganovic et al. 2011). Beganovic et al. (2011) conducted research on spontaneous sauerkraut fermentation, using 4% salt, and found that the pH did not drop below 4.6 until day 28. Interestingly, the mean pH values for WK and SK treatments were similar at the start of the study and the end. For WK treatments, the starting pH was 5.8 the end pH was 3.9. For SK treatments, the mean pH was 5.5 at the beginning of the study and 4.0 at the end.

Table 4. Mean pH values of seaweed sauerkraut samples over time.

Species	Sugar Kelp			Winged Kelp		
Concentration (% seaweed)	**25**	**50**	**75**	**25**	**50**	**75**
Day 0	5.66± 0.22	5.38± 0.18	5.39± 0.07	5.79± 0.17	5.75± 0.08	4.21± 0.32
Day 1	6.18± 0.03	5.87± 0.08	5.63± 0.07	6.13± 0.13	5.87± 0.15	5.56± 0.03
Day 3	5.78± 0.07	5.74± 0.07	4.85± 0.09	4.40± 0.13	4.52± 0.20	5.56± 0.03
Day 5	4.52± 0.99	5.92± 0.09	4.97± 0.35	3.55± 0.03	3.63± 0.03	3.99± 0.23
Day 7	4.47± 0.92	5.22± 1.04	5.10± 0.31	3.45± 0.02	3.49± 0.11	4.17± 0.10
Day 14	3.38± 0.08	3.52± 0.27	4.56± 0.35	3.38± 0.01	3.39± 0.04	4.23± 0.04
Day 28	3.42± 0.08	3.56± 0.31	3.81± 0.54	3.45± 0.02	3.47± 0.09	4.35± 0.06
Day 60	3.77± 0.11	3.88± 0.25	4.24± 0.44	3.73± 0.04	3.74± 0.04	4.21± 0.32

Values are means ± S.D. (n=3).

3.4. Titratable acidity

Similar to the pH results, there was a significant interaction among species, seaweed concentration, and time ($p=0.02$). TA was significantly different among species, as the overall TA of 0.31% for SK samples was significantly ($p<0.01$) lower than the TA of 0.41% for WK. This was expected because TA is typically higher in samples that have a lower pH, as supported by the significant ($p<0.01$) strong negative correlation (-.848) between TA and pH (Table 5). TA was positively correlated with aerobic plate counts and lactic acid bacteria, meaning that the TA increased when the lactic acid bacteria and aerobic plate count increased ($p<0.01$).

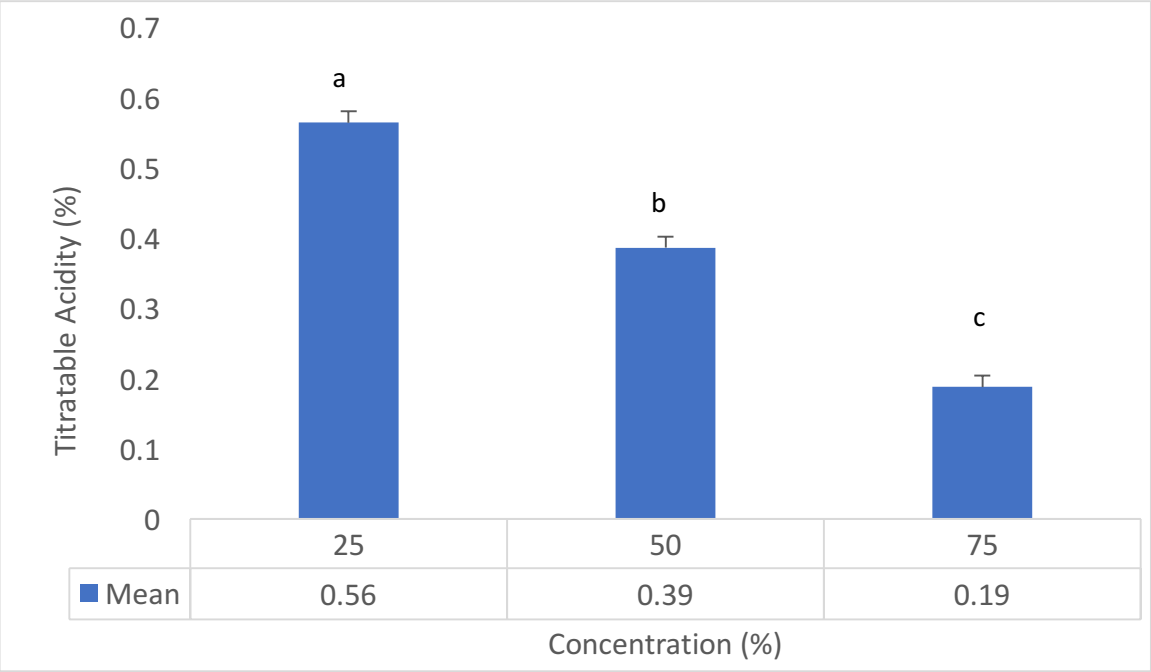
Table 5. Spearman correlations among pH, titratable acidity (TA), lactic acid bacteria (LAB) population, and aerobic plate counts (APC).

	pH	TA	LAB	APC
pH	____	-.848**	-.353**	-.296***
TA	-.848**	____	.266**	.259**
LAB	-.353**	.266**	____	.850**
APC	-.296***	.259**	.850**	____

**=$p<0.01$, ***=$p<0.001$.

Overall, as the concentration of seaweed increased, the TA decreased significantly ($p<0.01$) at each concentration level (Figure 9). Specifically, this was the case for the SK treatments. However, in WK treatments, the 25% and 50% TA were not significantly different, but both were significantly lower than the 75% treatment ($p<0.01$).

Figure 9. Overall mean titratable acidity (%) in seaweed sauerkraut by seaweed concentration.



Values are means ± S.E. Values not sharing a letter are significantly ($p<0.001$) different from each other based on Multi-Way ANOVA, followed by a Tukey's HSD post hoc test (n=6).

Among all treatments, time had significant ($p<0.01$) interactions with percent, as well as seaweed species ($p<0.01$). Over time, the average TA increased significantly ($p<0.01$) from the beginning of the study at 0.05% (Day 0) to 0.67% (Day 60) (Table 6).

Table 6. Mean titratable acidity (%) in seaweed sauerkraut over time.

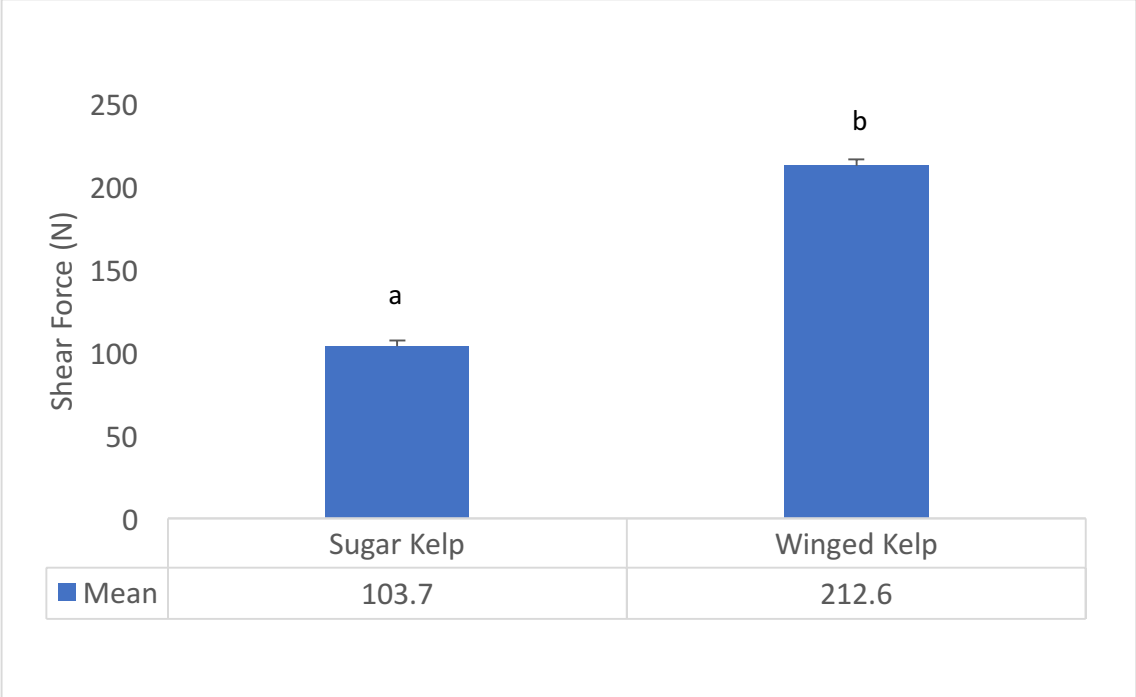
Species		**Sugar kelp**			**Winged Kelp**		
Concentration (% Seaweed)		25	50	75	25	50	75
Days	**0**	0.04± 0.01	0.05± 0.01	0.05± 0.01	0.04± 0.01	0.06± 0.02	0.04± 0.01
	1	0.04± 0.00	0.05+ 0.00	0.07± 0.00	0.05± 0.00	0.04± 0.00	0.06± 0.00
	3	0.07± 0.02	0.05+ 0.03	0.08± 0.01	0.23± 0.03	0.18± 0.02	0.18± 0.01
	5	0.14± 0.11	0.05± 0.01	0.09± 0.01	0.52± 0.06	0.41± 0.07	0.17± 0.01
	7	0.44± 0.23	0.16± 0.11	0.11± 0.02	0.92± 0.03	0.75± 0.09	0.27± 0.06
	14	1.07± 0.12	0.68± 0.37	0.15± 0.01	1.11± 0.03	0.90± 0.07	0.36± 0.11
	28	1.26± 0.17	0.77± 0.34	0.34± 0.12	1.03± 0.02	0.78± 0.02	0.44± 0.22
	60	1.04± 0.09	0.54± 0.24	0.21± 0.06	1.02± 0.08	0.73± 0.07	0.39±0.12

Values are means ± S.D (n=3).

3.5. Texture analysis

Texture of fermented products is a key attribute for consumers because it is important that the structure of the product is preserved. Kelp species significantly ($p<0.001$) affected the mean shear force (N) values in the sauerkraut throughout the study (Figure 10). WK treatments were ~2 times higher in mean shear force values than SK treatments throughout the study. This result was expected because WK has a distinct midrib in the middle of its blade. This midrib is thicker than the surrounding blade and similar in thickness to seaweed stipes. This likely accounted for the higher resistance to shear when comparing the two species, because SK has a thinner undivided blade.

Figure 10. Mean shear force (N) of seaweed sauerkraut by species.

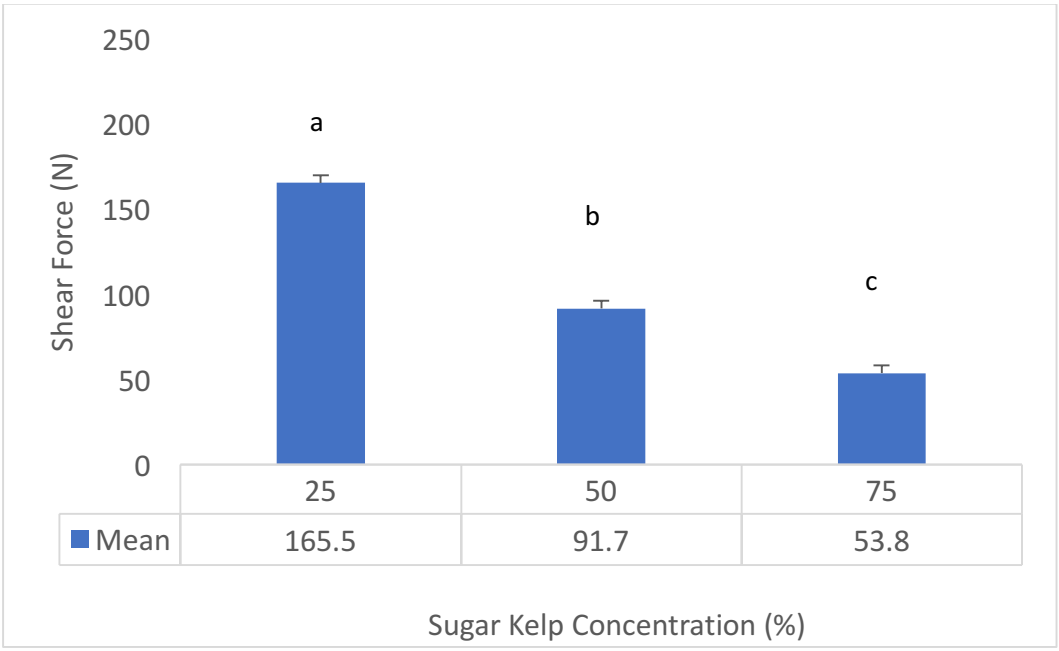


Values are means ± S.E. Values not sharing a letter are significantly ($p<0.001$) different based on Multi-Way ANOVA (n=9).

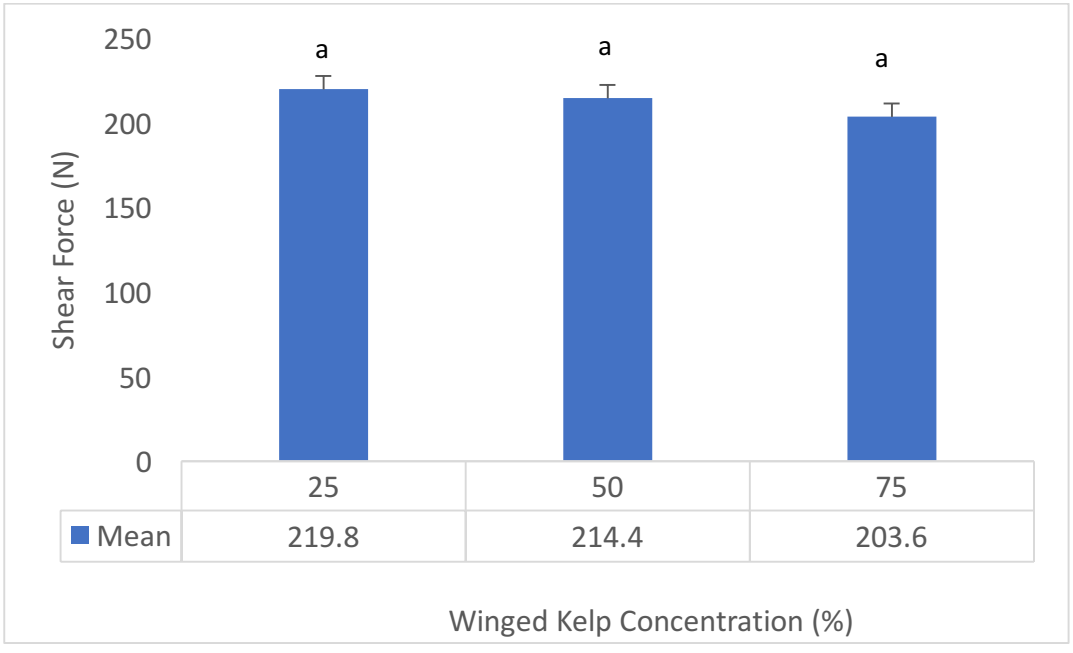
There was a significant ($p<0.01$) interaction between the seaweed species and seaweed concentration texture values. The texture of the SK sauerkraut treatments was significantly influenced by seaweed concentration. For SK treatments, as concentrations of SK increased from 25% to 75%, shear force decreased significantly from 165.5 to 53.8 N (Figure 11a). Interestingly, the percent incorporation did not influence the shear force values of the WK sauerkraut (Figure 11b), indicating that WK texture was similar to cabbage in terms of resistance to shear.

Figure 11. Mean shear force (N) of seaweed sauerkraut by seaweed concentration, a) sugar kelp and b) winged kelp.

a)



b)



Values are means ± S.E. Values not sharing a letter are significantly ($p<0.05$) different from each other based on Multi-Way ANOVA followed by a Tukey's HSD post hoc test (n=3).

Throughout the study, there was a significant interaction ($p<0.01$) between seaweed species and time. The WK and SK treatments showed similar trends over time with the lowest shear force values recorded at the beginning of the study and the highest values on day 27, indicating that resistance to shear increased significantly over time for the first half of the storage period (Table 7). The mean shear resistance of the SK and WK treatments significantly decreased by over 30 percent from day 27 to the end of the study (Day 60) with mean values changing from 208.5 N on day 27 to 142.7 N on day 60. The increase in shear resistance found in the first half of this study could be due to the use of starter cultures. Johanningsmeier et al. (2007) found that the firmness of spontaneous fermentation products varied within treatments and decreased over time. Yet, the addition of *L. mesenteroides*, a lactic acid bacteria starter culture, to the sauerkraut helped to retain the texture. The addition of salt to the treatments could have also influenced the shear resistance values found in the seaweed sauerkraut. Johanningsmeier et al. (2007) found that adding salt increased the crunchiness (shear firmness) of conventional cabbage sauerkraut. Their study suggested that salt inhibited activity of softening enzymes and preserved the original texture (Johanningsmeier et al. 2007). This could explain why the average shear firmness among all the treatments did not decrease until day 60 for this seaweed sauerkraut study.

Table 7. Mean shear force values (N) in seaweed sauerkraut over time.

Species	Concentration (% seaweed)	Days			
		6	12	27	60
Sugar Kelp	25	125.5 ± 37.3	140.5 ± 32.4	209.9 ± 29.1	186.2 ± 29.6
	50	61.5 ± 19.9	86.9 ± 23.9	111.7 ± 19.0	106.8 ± 21.7
	75	34.2 ± 14.1	38.1 ± 15.2	88.7 ± 17.1	54.0 ± 14.6
Winged Kelp	25	172.1 ± 37.9	249.4 ± 34.4	278.6 ± 40.7	179.2 ± 40.5
	50	164.7 ± 7.9	266.3 ± 6.7	267.7 ± 7.1	158.9 ± 42.5
	75	127.9 ± 48.5	221.0 ± 49.1	294.3 ± 47.4	171.1 ± 41.1

Values are means ± S.D. (n=3).

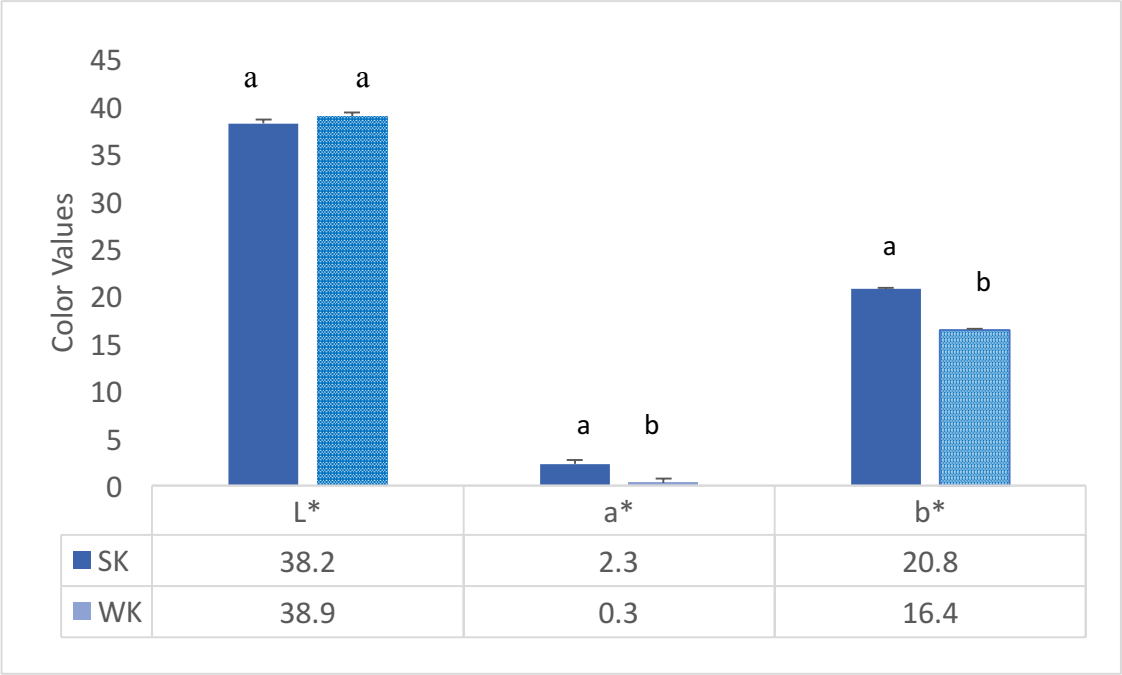
3.6. Colorimetric analysis

Color is an indicator of quality for many food products and measuring color can show how the products change over time. Regarding species, the L* values were not significantly different between WK and SK samples (Figure 12). However, a* and b* values were both significantly ($p<0.01$) higher in SK sauerkraut treatments. For b* values, there was also a significant ($p<0.01$) three-way interaction between seaweed species, seaweed concentration, and time. These data indicate that SK samples were more red and yellow in color than the WK treatments. It could also suggest that SK treatments had more fucoxanthin, as this pigment is responsible for the yellowish and brownish color of seaweeds (Mouritsen et al. 2013b). When fuxoxanthin is present, the pigment responsible for green color, chlorophyll a, is masked. WK treatments were greener than SK treatments, indicating that chlorophyll a was a more dominant pigment.

The difference in dominant pigments among species could impact the marketability and consumers' response to this product. For example, consumers typically relate the color green to

nature (Sliburyte and Skeryte 2014). The greener WK might be considered more favorable to consumers wanting more natural products, although, both products are equally natural.

Figure 12. Mean L* a* b* values of seaweed sauerkraut samples by species.



	L*	a*	b*
SK	38.2	2.3	20.8
WK	38.9	0.3	16.4

Values are means ± S.E. Values not sharing a letter are significantly different ($p<0.01$) from each other based on Multi-Way ANOVA followed by a Tukey's HSD post hoc test ($n=9$).

There was a significant interaction between seaweed concentration and seaweed species for L* values. As the seaweed concentration increased the L* values decreased significantly ($p<0.01$). This was expected because the seaweed was darker than the cabbage. The a* values decreased as the seaweed concentration increased, indicating that the sample became greener with the addition of seaweed. For a* and b* values, there was a significant interaction between seaweed concentration and time ($p=0.02$, $p=0.05$). A significant interaction ($p=0.05$) also occurred between seaweed concentration and species ($p=0.05$) in the b* values, as the b* values were highest in the 25%, yet lowest in the 50% concentrations (Table 8).

Table 8. Mean L*, a*, and b* values in seaweed sauerkraut over time.

Species			Sugar Kelp			Winged Kelp		
Concentration (% Seaweed)			25	50	75	25	50	75
Days	**6**	**L***	43.92 ± 3.69	32.66± 1.12	28.73± 1.09	40.49 ± 1.50	38.93± 1.01	29.58± 3.47
		a*	-0.02 ± 0.25	0.23± 1.03	2.00± 0.32	3.80± 0.76	3.26± 0.54	3.79± 0.70
		b*	18.62 ± 0.87	15.40 ± 1.31	17.09± 0.30	22.90± 1.13	21.74± 0.77	21.00± 1.39
	12	**L***	46.62 ± 2.29	39.80± 0.92	32.17± 2.21	42.18± 2.21	38.79± 2.26	30.99± 1.45
		a*	-0.35± 0.34	-0.41± 0.42	2.02 ± 0.83	0.56± 0.81	1.45± 0.43	4.31± 0.21
		b*	17.86± 1.08	14.16± 0.71	15.31± 0.47	20.53± 1.21	19.09± 0.40	22.44± 0.60
	27	**L***	47.06 ± 3.97	39.97± 3.00	33.68± 1.93	49.00± 2.44	39.60± 1.71	32.62± 1.22
		a*	-0.40 ± 0.09	-0.35± 0.42	1.38± 0.76	0.76± 0.68	0.72± 0.50	2.34± 0.95
		b*	17.84 ± 0.47	15.47± 0.25	14.54 ±1.02	21.02± 1.15	18.90± 0.50	20.36± 1.47
	60	**L***	48.66± 2.91	37.57±1.58	36.47 ± 5.07	47.04± 4.63	38.84± 0.95	32.66± 0.67
		a*	-0.68 ± 0.38	-0.77± 0.38	1.12 ± 0.75	0.79± 1.65	1.00± 0.36	3.90± 0.76
		b*	18.14 ± 2.27	15.13± 0.23	17.75± 0.30	21.30± 1.22	18.41± 0.45	20.67± 0.45

Values are means ± S.D. (n=3).

Additionally, the overall L* values were significantly lower on day 6 than other days of the study. After day 6, the L* values stayed consistent for the rest of the storage time. This indicates that the samples color was maintained. Additionally, a* and b* values were highest on day 6.

3.7. Antioxidant analysis

3.7.1. Total phenolic content (TPC)

Throughout the study, SK and WK treatments had very similar average total phenolic contents (TPC) of 2.38 (mg/g) and 2.61(mg/g), respectively, indicating that species did not influence TPC. The similarity in TPC among WK and SK sauerkraut treatments is interesting because other researchers have reported significant differences in TPC among various seaweed species (Gupta et al. 2012). Gupta et. al (2012) compared the TPC among two different kelp species, *Laminaria digitata* and *Saccharina latissima* (SK). The TPC for *L. digitata* was 3.1 times lower than for the SK. The variability found among species in that study may have been due to environmental and location factors. Therefore, the similar TPC found among WK and SK sauerkraut treatments could be because they were harvested at the same time and in the same location.

As seaweed concentration increased in SK and WK sauerkraut samples, TPC also increased and there was a significant ($p<0.01$) interaction among seaweed concentration and seaweed species (Table 9). The 75% treatments were more than 50% higher in TPC than the 25% seaweed sauerkraut. However, the TPC in 25% and 50% treatments were not significantly different from each other for both the WK and SK treatments. Comparing the TPC in this study to a red cabbage sauerkraut study by Hunaefi et al. (2013) suggests that the addition of seaweed may have increased the TPC of the sauerkraut. The TPC in red cabbage inoculated with *L.*

plantarum ranged from 1.29-1.85 mg/g throughout a 38-day study, almost 30% lower than the average TPC found in the WK (2.61 mg/g) and SK (2.38 mg/g) treatments. However, we cannot confirm that the seaweed was directly responsible for higher TPC level between studies, since different cabbage species were also used. Yet, TPC of seaweed sauerkraut can be directly compared to the TPC of fresh WK and SK blades. Nayyar (2016) reported that the average TPC of WK and SK were approximately 14 mg/g and 2 mg/g, respectively. This suggests that SK and SK sauerkraut are similar in TPC. However, Nayyar (2016) recorded TPC levels in WK more than 3x higher than levels found in 75% WK sauerkraut. Differences among studies may be due to seasonality and the fact that seaweed sauerkraut treatments had less seaweed, however, this would only account for a slight variation. The TPC of the seaweed sauerkraut treatments did not significantly change during storage. Additionally, these TPC values can be compared to berries, fruits well-known for their high antioxidant activity. Huang et al. (2012) found that blueberries, strawberries, and blackberries had a TPC of 9.44, 2.72, and 5.58 mg/g, respectively. While blueberries had a particularly high TPC, the TPC of seaweed sauerkraut was comparable to those of strawberries and blackberries, indicating that this product also has high antioxidant activity.

Table 9. Mean total phenolic content (mg/g) of seaweed sauerkraut over time.

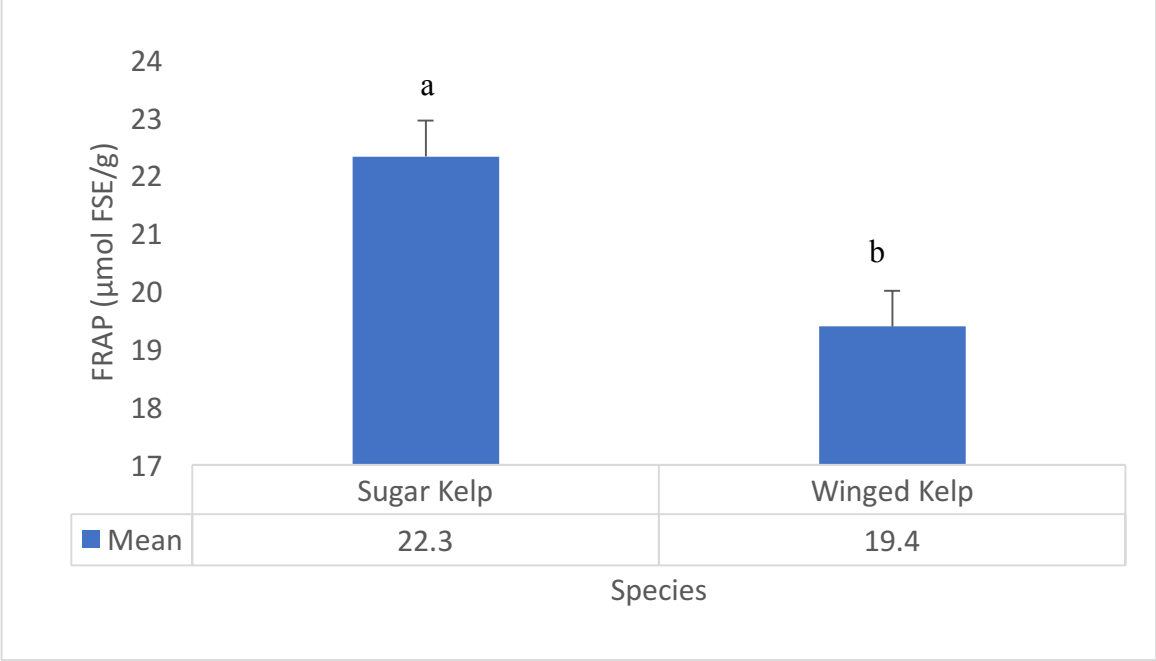
		Days		
Species	**Concentration (% seaweed)**	**21**	**42**	**60**
Sugar Kelp	25	1.78 ± 0.08	1.91 ± 0.11	2.11 ± 0.14
	50	2.20 ± 0.29	2.17 ± 0.14	2.12 ± 0.15
	75	2.49 ± 0.5	3.43 ± 0.36	3.20 ± 0.36
Winged Kelp	25	1.58 ± 0.06	1.78 ± 0.1	1.83 ± 0.15
	50	1.68 ± 0.29	2.04 ± 0.15	2.01 ± 0.30
	75	3.90 ± 0.46	4.48 ± 0.88	4.17 ± 0.52

Values are means ± S.D. (n=3).

3.7.2. Ferric reducing antioxidant power (FRAP) assay

The FRAP assay assesses the ability of an antioxidant to reduce reactive species. It is an electron transfer reaction that reduces a ferric oxidant to a ferrous ion (Benzie and Strain 1996). The higher FRAP values found in SK sauerkraut show that species did influence FRAP values and that SK treatments had higher antioxidant capacity (Figure 13). Seaweeds tend to differ in FRAP values among species, with brown seaweeds having high FRAP values when compared to red seaweeds (Matanjun et al. 2009; O'Sullivan et al. 2011). Furthermore, fermented products have shown higher FRAP values than non-fermented products (Cho et al. 2015). One reason for this could be because fermentation releases active peptides, due to the use of *Lactobacillus*. A study by Ramesh et al. (2012) found that *Lactobacillus* strains produced bioactive peptides in milk, which have antioxidant activity due to their interaction with free radicals.

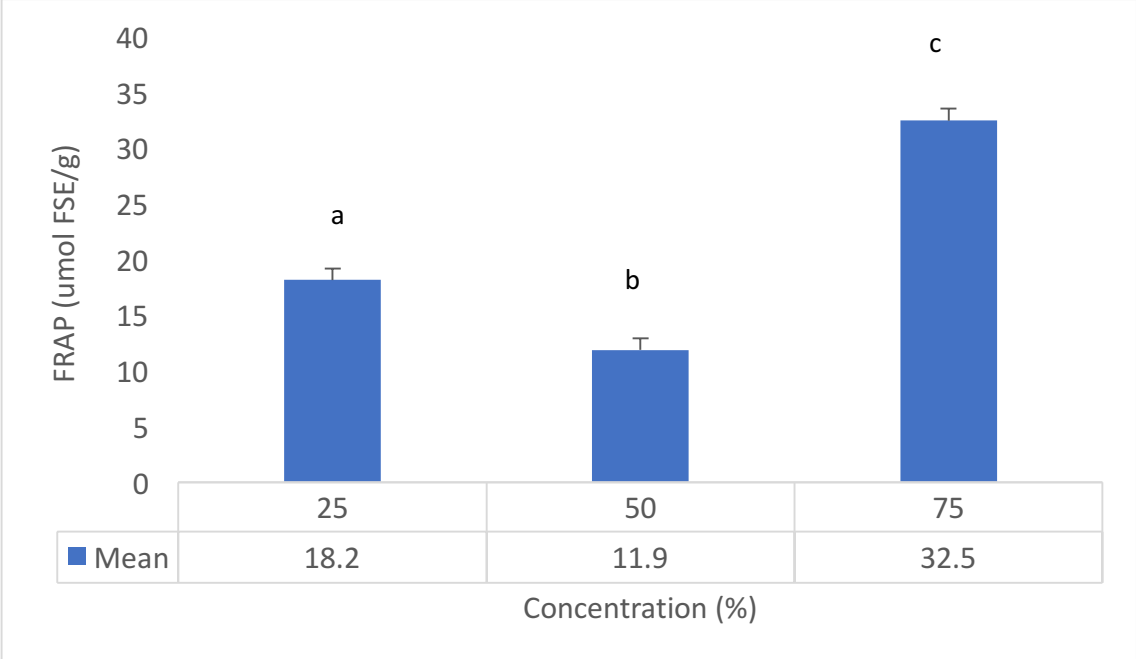
Figure 13. Overall mean FRAP values (µmol FSE/g) in seaweed sauerkraut by species.



Values are means ± S.E. Values not sharing a letter are significantly ($p<0.05$) different from each other based on Multi-Way ANOVA followed by a Tukey's HSD post hoc test (n=9). FRAP = ferric reducing antioxidant power.

Similar to the results of the TPC analysis, the percentage of seaweed significantly affected the FRAP values. There was a significant interaction between seaweed species and seaweed concentration ($p<0.01$). The highest FRAP values were found in the 75% treatments, with an average of 26.6 µmol FSE/g dried seaweed sauerkraut in the 75% WK treatment and 37.4 µmol FSE/g in the 75% SK treatment. Additionally, the 75% SK FRAP values were 54% higher than the 25% treatments. In WK, the 75% FRAP values were 30% higher than the 25% treatments (Figure 14). Unexpectedly, the FRAP values were lowest in the 50% treatments for both the SK and WK.

Figure 14. Overall mean FRAP values (µmol FSE/g) in seaweed sauerkraut by seaweed concentrations.



Data are means ± S.E. Values not sharing a letter are significantly ($p<0.05$) different from each other based on a Multi-Way ANOVA followed by a Tukey's HSD post hoc test (n=6). FRAP = ferric reducing antioxidant power.

Overall there was a three-way interaction ($p<0.01$) between time, seaweed concentration, and seaweed species for FRAP values. There was also a two-way interaction between time and

seaweed species ($p=0.01$) and time and seaweed species. ($p<0.01$). Time influenced the FRAP values, as the values in SK treatments were 10% higher at day 60 compared to day 21 (Table 10). Comparably WK treatments were approximately 80% higher at day 60 than day 21. The increase in FRAP values was mostly due to the 75% sauerkraut treatments. The increase in FRAP values over time could be due to fermentation and the presence of lactic acid bacteria, as previously mentioned. In this study, the overall average lactic acid bacteria counts were lowest on day 21 and highest on day 60. Therefore, the increase in lactic acid bacteria metabolic activity could have produced compounds that increased the antioxidant activity.

Table 10. Mean FRAP (μmol FSE/g) values in seaweed sauerkraut over time.

Species	Concentration (% seaweed)	Days		
		21	42	60
Sugar Kelp	25	17.4 ± 3.4	13.0 ± 4.7	20.0 ± 2.6
	50	9.2 ± 0.5	16.9 ± 1.9	11.4 ± 0.9
	75	36.0 ± 3.6	38.7 ± 1.9	37.4 ± 2.5
Winged Kelp	25	18.2 ± 1.8	18.4 ± 3.1	19.4 ± 3.1
	50	10.4 ± 0.5	11.5 ± 0.8	10.6 ± 1.1
	75	9.4 ± 1.0	39.2 ± 5.6	37.5 ± 5.9

Values are means ± S.D. (n=3). FRAP = ferric reducing antioxidant power.

3.7.3. α, α-diphenyl-β-picrylhydrazyl (DPPH) assay

The results of the DPPH assay show that species did not influence the DPPH values. The DPPH units are reported in EC_{50} (mg/mL), which is the sample concentration (mg/mL) needed to inhibit 50% of the DPPH free radicals. Throughout the study, SK and WK treatments had very similar average EC_{50} values of 5.31 (mg/mL) and 5.54 (mg/mL), respectively.

As seaweed concentrations in the sauerkraut increased, EC_{50} values decreased significantly (Table 11). The lower the EC_{50} value of the sample, the higher its antioxidant capacity. The 75% treatments had the lowest EC_{50} values, while the highest was found in the 25% treatments. The 75% percent treatments were approximately three times lower than the 25% seaweed sauerkraut treatments, overall indicating that they had a higher antioxidant capacity. Data from Nayyar (2016) support the claim that the addition of seaweed contributes to higher antioxidant activity. Nayyar (2016) found low DPPH values in seaweeds, with an average EC_{50} ~7mg/mL in sugar kelp and a better value of ~1 mg/mL for winged kelp. For sauerkraut, this study found average EC_{50} values of 3.1 and 2.3 mg/mL, in the 75% SK and 75% WK treatments, respectively. While the WK EC_{50} values found in seaweed sauerkraut are comparable to those in raw WK, the SK EC_{50} values were lower (better) compared to raw sugar kelp. As discussed previously, the process of fermentation may have contributed to the lower EC_{50} values by increasing the production of antioxidant metabolites.

Table 11. Mean DPPH EC_{50} (mg/mL) values in seaweed sauerkraut over time.

Species	**Concentration (% seaweed)**	**Days**		
		21	**42**	**60**
Sugar Kelp	25	8.93± 0.86	7.70 ±1.70	5.73 ± 0.94
	50	6.32 ± 1.20	4.86 ±1.39	4.80 ± 1.24
	75	3.41± 1.39	2.94 ± 0.78	3.07 ± 0.53
Winged Kelp	25	7.96 ± 0.97	6.73± 0.99	7.52 ± 2.86
	50	7.44 ± 1.65	6.90 ±0.42	6.54 ± 0.76
	75	2.13 ± 0.57	2.67 ± 1.55	2.01 ± 0.56

Values are means ± S.D. (n=3). DPPH = α, α-diphenyl-β-picrylhydrazyl.

The DPPH EC_{50} values decreased somewhat over time, but the changes were not significant. Mean SK and WK EC_{50} values decreased from 6.22 to 4.53 mg/mL and 5.84 to 5.36 mg/mL, respectively, from day 21 to day 60 of the study. These results are comparable to those for FRAP values, as there was an increase in antioxidant capacity over time among sauerkraut samples.

Because TPC, FRAP, and DPPH assays are all indicators of antioxidant activity, they were compared using Spearman Correlations. DPPH EC_{50} values were strongly negatively correlated (-0.788, $p<0.01$) with TPC and to a lesser extent with FRAP (-0.541, $p<0.01$) (Table 12). FRAP and TPC were strongly positively correlated (0.601, $p<0.01$), meaning that samples with a higher TPC also had a higher FRAP. The data are supported by previous studies that also reported a high correlation between TPC and antioxidant activity among seaweeds (Chew et al. 2008, Rajauria et al. 2010). One reason for this high antioxidant activity is because seaweeds have a unique chemical composition. Seaweeds are rich in phlorotannins, which are the dominant phenolic compounds in brown seaweed. Seaweeds with more phlorotannins have showed high antioxidant activity. Brown seaweeds also have more biologically active compounds that have been known to inhibit enzymes (e.g. α-glucosidase, acetylcholinesterase, and butyrylcholinesterase) and free radicals, making the species rich in important antioxidants (Andrade et al. 2013). Additionally, seaweed is high in polysaccharides and these polysaccharides have sulphate groups that could enhance their antioxidant activities (Roohinejad et al. 2017).

Table 12. Spearman Correlation among TPC, DPPH, and FRAP values of seaweed sauerkraut.

	FRAP	DPPH	TPC
FRAP	____	-.541**	.601**
DPPH	-.541**	____	-.788**
TPC	.601**	-.788**	____

**=p<0.01, TPC = total phenolic content, DPPH = α, α-diphenyl-β-picrylhydrazyl, FRAP = ferric reducing antioxidant power.

3.8. Organic Acids

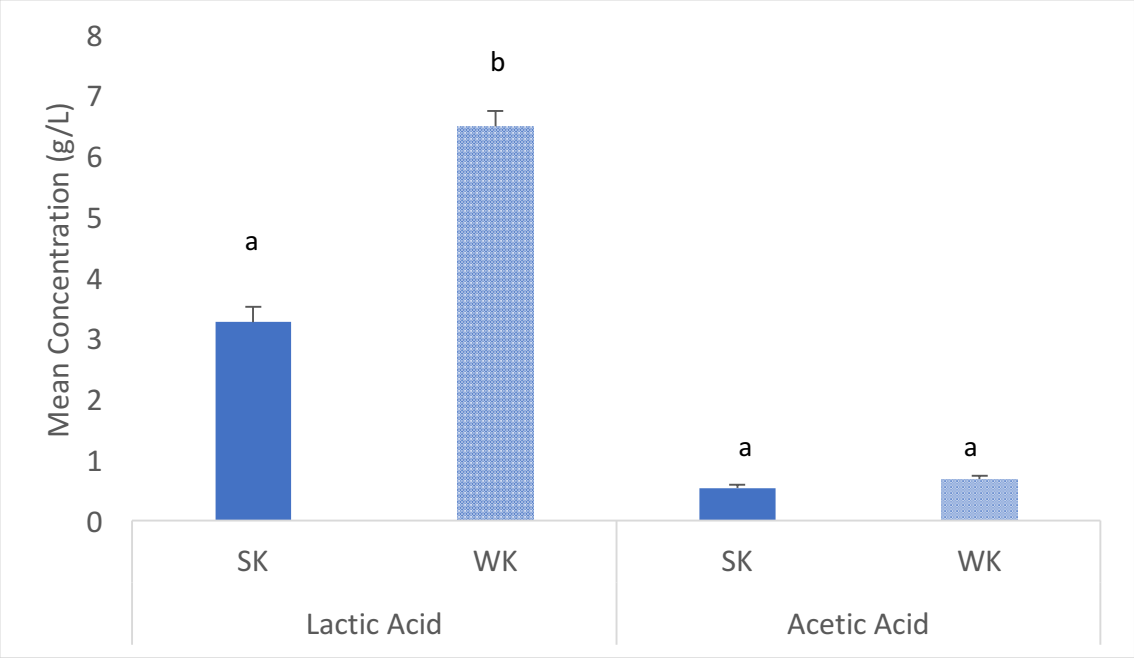
3.8.1. Lactic acid

Lactic acid is one of the main products produced by lactic acid bacteria during fermentation. Therefore, measuring the lactic acid concentration indirectly measures the progress of the fermentation. Furthermore, the growth of lactic acid bacteria helps create an acidic environment that deters spoilage bacteria. Also, it is responsible for the unique flavor that consumers identify in lacto-fermented products.

There was a significant ($p<0.01$) three-way interaction between all the tested variables and lactic acid. WK treatments had approximately double the amount of lactic acid concentration than SK treatments (Figure 15). Seaweed species and seaweed concentrations had a significant ($p<0.01$) interaction with the lactic acid concentrations and the 75% treatments had the lowest lactic acid concentrations. Additionally, lactic acid concentrations had significant interactions between time and seaweed species ($p<0.01$), time and seaweed concentrations ($p<0.01$). The lactic acid concentrations in the seaweed sauerkrauts were measured until day 14 and during this time, WK had higher recorded lactic acid bacteria counts and lower pH values than the SK treatments. These results confirm that the higher LAB populations created more lactic acid and

reduced the pH of the sauerkraut. SK took longer to ferment likely explaining the significantly lower lactic acid concentration.

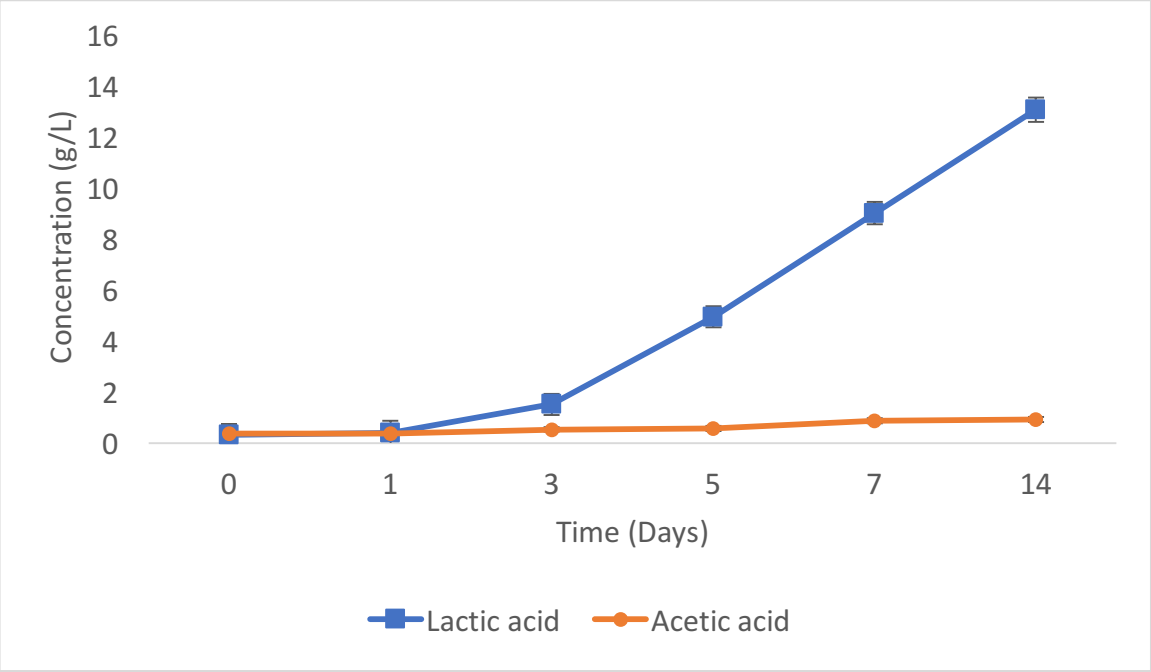
Figure 15. Mean lactic acid and acetic acid concentrations (g/L) in seaweed sauerkraut by species.



Values are means ± S.E. Values not sharing a letter are significantly different ($p<0.01$) from each other based on a Multi-Way ANOVA followed by Tukey's HSD post hoc test ($n=9$).

Lactic acid concentrations increased continually over time with the highest concentration found on day 14 (Figure 16). Overall, lactic acid was 40 times higher on day 14 day than on day 0. Lactic acid bacteria counts were highest on day 7 and remained high until day 14, suggesting that the growth of LAB effected the lactic acid concentrations.

Figure 16. Mean lactic acid and acetic acid concentrations (g/L) in seaweed sauerkraut over time.



Values are means ± S.E (n=18).

The lactic acid concentrations found in this study are comparable to another sauerkraut study. Trail et al. (1995) tested eight commercial sauerkrauts and found that lactic acid values ranged from 111-178mM (~10-16g/L) in the fermented products. These lactic acid concentrations are similar to the seaweed sauerkraut, which had a mean lactic acid concentration of 13 g/L at the end of the fermentation period.

3.8.2. Acetic acid

Acetic acid was present in low amounts in all treatments. This could be because *Lactobacillus plantarum* bacteria are facultative heterofermentors, meaning they can produce low levels of acetic acid and other products (FAO, 1998), in addition to lactic acid. Also, there could have been low levels of acetic acid bacteria present in the natural flora. Despite the low levels, there was a significant ($p=0.03$) interaction between all the tested variables with regard to

acetic acid concentrations. There was also a significant interaction ($p=0.03$) between seaweed concentration and seaweed species. Acetic acid production was highest in the 75 WK treatments. Regarding time, there was a significant ($p<0.01$) increase in mean acetic acid concentrations over time from day 0 (0.37 g/L) compared to day 14 (0.92 g/L).

3.9. Sugars

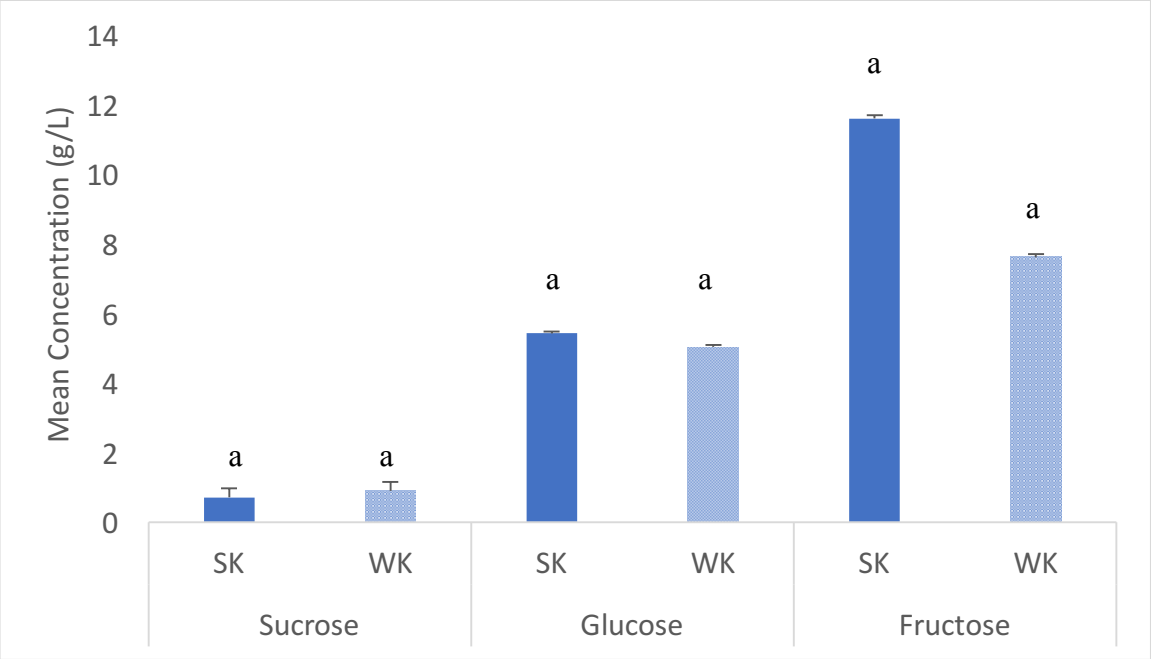
Sugars are one of the main carbohydrate sources that drive fermentation. Therefore, measuring the sugar concentrations in the brine can help clarify how and which carbohydrates are utilized by microorganisms.

The sugar concentrations among treatments were relatively similar between species, suggesting that the microorganisms utilized sugars similarly in fermented WK and SK products (Figure 17). In contrast, the concentration of seaweed influenced sugar concentrations in the sauerkraut treatments. Fructose, sucrose, and glucose were significantly higher in concentration in the 25% treatments and the lowest in the 75% treatments, likely due to the contribution from the cabbage fraction of the sauerkraut. Overall, cabbage has a higher sugar content than seaweed. The content of sugars in green cabbage is about 3.2 g per 100 g, while sugar kelp has 0.6 g per 100 g (USDA, 2018). In a study by Hughes and Lindsay (1985), it was found that glucose was the most abundant sugar in cabbage when compared to fructose and sucrose. Fructose is also an abundant sugar, while sucrose is typically found in small amounts in cabbage (Xiong et al. 2014).

Brown seaweeds are rich in polysaccharides and sugar alcohols like laminarin, alginate, mannitol and cellulose (Schiener et al. 2015). Laminarans are storage compounds and structural polysaccharides, monosaccharides linked with different glucosidic bonds, that are main components in the cell wall of seaweed (Perez et al. 2016). Laminarans can make up to 32% dry

weight (Kraan, 2012) of some brown seaweeds. Alginates are also structural polysaccharides and are major components of brown seaweed and are made up of mannuronic and guluronic acids (Perez et al. 2016). Cellulose is a cell wall polysaccharide that is insoluble (Kraan, 2012). Mannitol is a sugar alcohol found in brown seaweeds and in some species of brown seaweed it can make up 25% of the dry weight depending on the season (Kraan, 2012). Mannitol is used as a flavor enhancer and is known for its sweet flavor. These different carbohydrates are responsible for some of the diverse sugars found in seaweed, including D-galactose, D-mannitol, L-rhamnose, D-glucuronic acid, and L-fucose (Hwang et al. 2011).

Figure 17. Mean sugar content (g/L) in seaweed sauerkraut by species

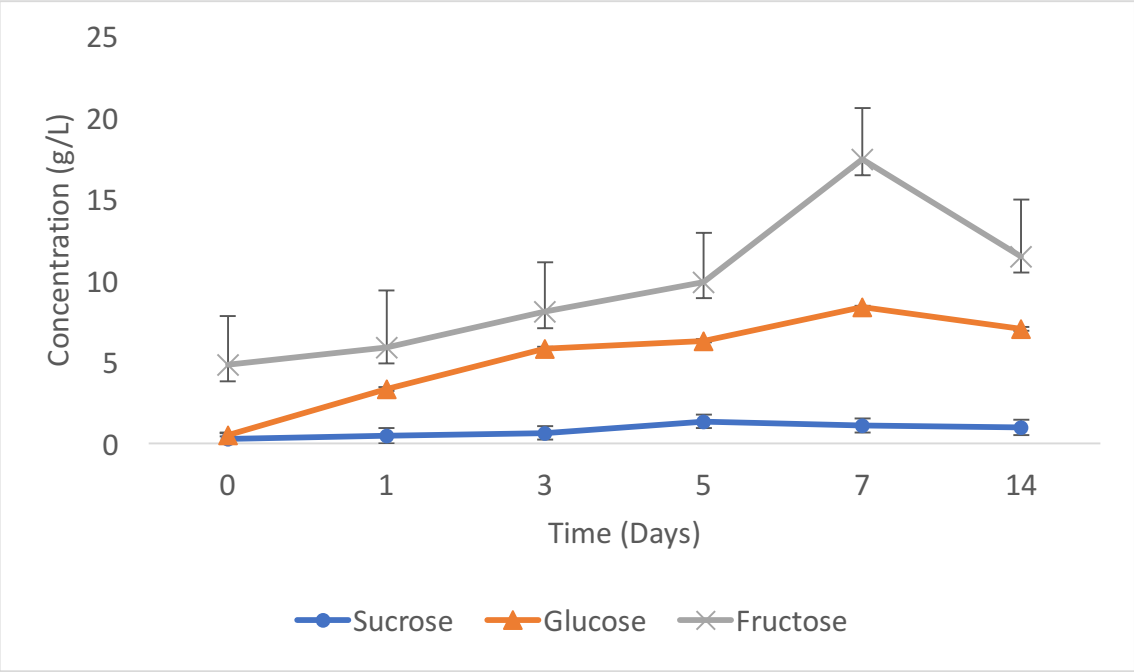


Values are means ± S.E. Values not sharing a letter are significantly different from each other based on Multi-Way ANOVA followed by Tukey's HSD post hoc test (n=9).

Sucrose content was negligible in the seaweed sauerkraut, however, fructose and glucose content increased over time, with the highest concentrations found on day 7 (Figure 18). The low sucrose concentration could suggest that sucrose was hydrolyzed, resulting in a slight increase in

glucose and fructose concentrations throughout the study (Xiong et al. 2014). Fructose levels increased approximately fourfold by day 7 and there was approximately two times as much fructose than glucose. Similarly, by day 7 glucose levels increased to 8.35 g/L, approximately 16 times the concentration on day 0 (0.52 g/L). The increase in fructose and glucose concentration could have been partially due to diffusion of these sugars from the cabbage into the brine. Overall, these results are comparable to a study by Trail et al. (1995) that tested eight commercial sauerkrauts and found that glucose ranged from 0-57mM (~0-10g/L), and fructose ranged from 0-22mM (0-4g/L). The somewhat higher fructose concentrations observed in the seaweed sauerkraut brine can further be explained by a study by Xiong et al. (2016) that measured different chemical compositions of sauerkrauts prepared with different salt concentrations. In that study, fructose concentrations were initially higher in the cabbage than in the brine. However, over time, the fructose concentration quickly increased in the brine and then remained steady throughout the fermentation (Xiong et al. 2016). That study suggested that the increase in fructose concentrations could also indicate that this sugar was not utilized by the microorganisms.

Figure 18. Overall mean of sugar concentration (g/L) in seaweed sauerkraut over time.



Values are means ± S.E. (n=18).

Furthermore, Hwang et al. (2011) conducted a study that compared the fermentation of the aforementioned seaweed sugars to common sugars found in land plants (D-glucose, D-xylose, L-arabinose, and D-mannose) by measuring lactic acid yields. They also compared different lactic acid bacteria strains and their lactic acid yield from different sugars. The authors reported that seaweed sugars could be fermented, and that different strains of lactic acid bacteria utilized seaweed sugars differently, resulting in varying lactic acid yields. D-mannitol showed the highest L-lactic acid yield (>10 g/L) among the seaweed sugars among five different lactobacillus strains, higher than lactic acid yields from D-glucose and D-galactose. However, D-glucose and D-galactose also produced high lactic acid yields, meaning that they were readily consumed by the lactic acid bacteria. Furthermore, this study found that *L. plantarum* produced the highest lactic acid yield (≈ 90%), suggesting this would be the best species to ferment the sugars (Hwang et al. 2011). Therefore, in the current study, the sugars from the seaweed (D-

galactose and D-glucuronic acid) could have influenced the sugar concentrations that were found in the seaweed sauerkraut brine. Evaluating the concentration of specific seaweed sugars and monitoring their changes during fermentation may provide insight into their potential influence on fermentation.

3.10. Sensory testing

Sensory evaluation was conducted on four different seaweed sauerkraut formulations to determine how consumers would react to this product. One hundred panelists participated in this study: 56% female and 44% male. A majority (71%) of the panelists were in the age range of 18-24 or 25-31. When asked how often they consumed seaweed, 70% indicated that they consumed seaweed either 1-4 times per year (32%) or 1-2 times per month (38%). Panelists consumed seaweed more often than sauerkraut, as most panelists consumed sauerkraut less than once a year (39%) or 1-4 times per year (38%). Over 56% of panelists consumed probiotics at least once a week and 73% of panelists were aware that fermented foods contained probiotics, indicating that probiotics were an important part of their diet.

While many attributes were tested, the results of this sensory study indicate that there were no significant differences among the four different seaweed sauerkraut treatments for all attributes (Table 13). The lack of significant difference in the sensory attributes among treatments was surprising because physicochemical analyses revealed significant differences among treatments. Although sourness was also tested, the data could not be used due to errors in the computerized testing procedure.

Table 13. Mean hedonic scores for four seaweed sauerkraut treatments on a 9-point hedonic scale.

Attribute	25 SK	50 SK	25 WK	50 WK	P-Value
Color	6.18 ± 1.49	6.15 ± 1.59	6.14 ± 1.31	5.86 ± 1.75	0.4302
Aroma	5.48 ± 1.77	5.40 ± 1.75	5.13 ± 1.79	5.30 ± 1.60	0.5213
Flavor	6.01 ± 1.85	5.81 ± 1.91	5.73 ± 1.88	5.79 ± 1.84	0.7401
Texture	6.73 ± 1.40	6.47 ± 1.51	6.65 ± 1.44	6.60 ± 1.34	0.6291
Overall Liking	5.99± 1.66	5.79 ± 1.80	5.82 ± 1.85	5.85 ± 1.74	0.8627

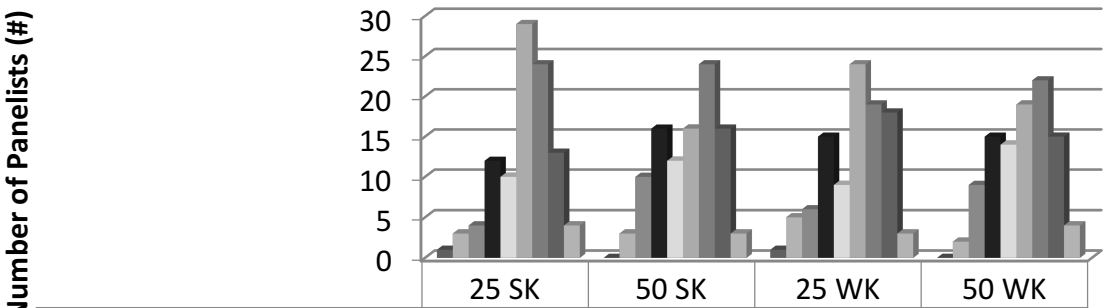
Values are means ± S.D. No significant differences among the means were found based on a Multi-way ANOVA (n=100).

3.10.1. Overall liking

Using the 9-point hedonic scale, the average "Overall liking" scores for these products was 5.86, just under 6.0 which is equivalent to "Like slightly." The "Overall liking" scores of consumers who ate seaweed more than once a year averaged 5.95, also equivalent to "Like slightly." Those who consumed seaweed less than once a year rated the "Overall liking" of the sample approximately half a point lower with an average of 5.52, suggesting that overall acceptability of the seaweed sauerkraut was related to the panelists' familiarity with seaweed products.

The overall distribution for "Overall liking" was bell curved, meaning that the data were normally distributed. A majority of panelists rated the samples at or above a 6, which is the "Like slightly" category (Figure 19). The 25% SK sample had the highest "Overall liking" score (5.99 ± 1.66), and 70% of panelists chose "Like slightly" or higher for this sample. The 50% SK treatment had the lowest "Overall liking" score (5.79 ± 1.80), with 59% of panelists rating the sample as "Like slightly" or higher.

Figure 19. Frequency of "Overall liking" scores for four seaweed sauerkraut treatments.



	25 SK	50 SK	25 WK	50 WK
Dislike Extremely (1)	1	0	1	0
Dislike Very Much (2)	3	3	5	2
Dislike Moderately (3)	4	10	6	9
Dislike Slightly (4)	12	16	15	15
Neither Like nor Dislike (5)	10	12	9	14
Like Slightly (6)	29	16	24	19
Like Moderately (7)	24	24	19	22
Like Very Much (8)	13	16	18	15
Like Extremely (9)	4	3	3	4

3.10.2. Flavor

Flavor is usually considered one of the most important attributes for product development. The 25% SK treatment had the highest mean score (6.01 ± 1.85) for flavor. Panelists commented that this sample had just the right amount of flavor and that it was good. Additionally, this sample had a nice pickle flavor that helped disguise the ocean flavor. Other panelists thought that this sample was too sour and didn't taste like sauerkraut. Some panelists thought that 50% SK tasted better and had more flavor than the 25% SK. The 25% WK was described as more bitter, sour, and too seaweedy/smelly. However, the 50% WK was described as having a pleasant seaweed flavor. Overall, consumers had varying opinions when describing the flavor of the samples that were both positive and negative, making it difficult to draw conclusions about flavor attributes.

All sensory attributes tested were significant and positively related (Table 14). Additionally, "Overall liking" and "flavor" were strongly related (0.89), showing that panelists who rated the sample higher for overall liking also liked the flavor of the sample. "Overall liking" and "texture" were also strongly related (0.63), meaning that panelists that liked the texture also liked the product more, overall. Panelists who rated the flavor higher seemed to like the aroma more, making these attributes strongly related (0.63).

Table 14. Spearman Correlation table of sensory attribute scores.

	Color	**Aroma**	**Flavor**	**Texture**	**Overall Liking**
Color	1	.506**	.498**	.422**	.495**
Aroma	.506**	1	.628**	.419**	.649**
Flavor	.498**	.628**	1	.593**	.892**
Texture	.422**	.419**	.593**	1	.632**
Overall Liking	.495**	.649**	.892**	.632**	1

**=p<0.01

3.10.3. Color

The results from instrumental color analysis indicated the seaweed sauerkrauts were significantly different in color, with SK samples being more red and yellow, while WK samples were more green and blue in color. In addition, seaweed samples were detectably different in color based on observation. The mean hedonic scores for color ranged from 5.86 to 6.18 among treatments. Panelists noted that they liked the vivid colors of the seaweed and disliked the bleached color of the cabbage. However, the panelists did not rate the acceptability of color differently among the samples.

3.10.4. Aroma

The aroma of seaweed and fermented products is unique. A majority of the panelists' comments were related to aroma. Despite this, there were no significant differences found among samples with regard to aroma. However, aroma had the lowest hedonic scores across all attributes with a mean range of 5.13-5.48 among samples, indicating that panelists had stronger reactions to this attribute. One reason for this is that panelists had different opinions on what they liked about the aroma. The **5**0% WK was described as having the best aroma by some panelists, while other panelists thought that the smell was too strong and ocean-y. Consumers commented that the 25% SK and 50% SK smelled nice. The 25% WK was described as having an off-putting smell that was not as good as the other samples.

3.10.5. Saltiness

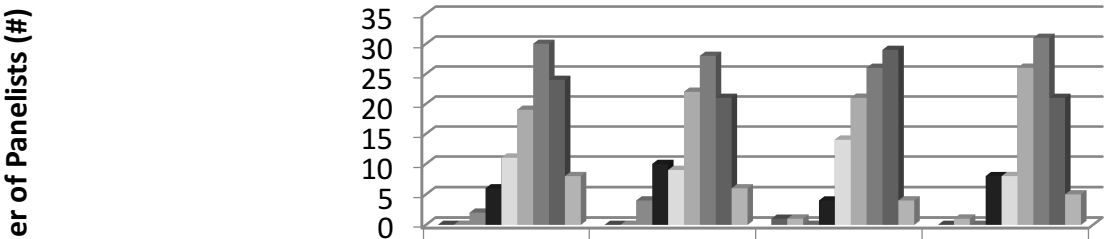
Seaweed grows in a saline environment and the process of making seaweed sauerkraut required adding more salt for preservation purposes. Panelists were asked to rate the salt intensity on a 5-point "Just about Right Scale" from 1 = "not nearly salty enough" to 5 = "much too salty." Overall, there were no significant differences in saltiness ratings among treatments. Consumers rated the amount of salt in the samples as "Just about right," with mean scores ranging from 2.92-3.14 among samples and an average mean for all the samples of 3.03 ± 0.63. The mean scores were surprising since a few panelists commented that all the samples, besides 25% SK, were too salty.

3.10.6. Texture

Texture is an important sensory property that was positively correlated with overall liking in the seaweed sauerkrauts. Panelists rated the texture scores highest among all hedonic scores. Panelists rated the texture similarly for each of the samples with mean scores ranging from 6.47-

6.73. This result is interesting because the shear force analysis indicated that there was a significant difference among seaweed species. SK treatments had lower shear force values, indicating softer texture. The higher shear force values in WK treatments could be from structural difference among the species because WK has a firm midrib on its blade. Differences in texture were also clearly apparent based on observation. While filling the sample cups, the mushier texture of the SK treatments was evident. Therefore, it was expected that panelists would rate the samples differently. Panelists described both the SK and WK samples as having a nice texture and crunch. The 25% SK sample was described as having a good seaweed to cabbage ratio. However, some panelists mentioned that the 50% SK treatment was slimy.

Figure 20. Frequency of texture scores for four seaweed sauerkrauts treatments.



	25 SK	50 SK	25 WK	50 WK
Dislike Extremely (1)	0	0	1	0
Dislike Very Much (2)	0	0	1	1
Dislike Moderately (3)	2	4	0	0
Dislike Slightly(4)	6	10	4	8
Neither Like nor Dislike (5)	11	9	14	8
Like Slightly (6)	19	22	21	26
Like Moderately (7)	30	28	26	31
Like Very Much (8)	24	21	29	21
Like Extremely (9)	8	6	4	5

Almost 80% of panelists rated the texture as “Like slightly” or higher for all the tested samples (Figure 20). The panelists’ frequency of choosing “Like slightly” or above ranged from 77%-83% among all samples. Overall, the hedonic scores for texture were highest among all attributes.

The hedonic scores for the seaweed sauerkraut samples could have been improved. Typically, sauerkraut is made with caraway seeds or other spices. Adding spices could have resulted in higher flavor scores and helped to mask some of the seaweed aroma that some panelists did not like. Also, sauerkraut is frequently consumed as a condiment along with sausages, not by itself. Furthermore, this product is something new that consumers have never tried before. This could have created a bias against the samples because panelists could have had preexisting expectations about how the product might, or should, have tasted. Additionally, the results may have been different if there had been more screening of consumers. Although we tried to recruit panelists who enjoy consuming seaweed and sauerkraut, if more panelists who frequently consume seaweed and sauerkraut had tested the product, the acceptability scores may have been higher.

4. OVERALL CONCLUSIONS AND RECOMMENDATIONS

This study is the first report in the scientific literature on lactic acid fermentation of seaweed to produce seaweed sauerkraut, with a specific focus on Maine seaweed species incorporated at various levels.

The results indicate that kelps can be fermented successfully and that seaweed species and incorporation level affect different attributes of the fermented products. Visually, the WK sauerkraut was slightly darker green and produced more brine. In the SK treatments, seaweed and cabbage were clumped together and were somewhat slimy to the touch. LAB grew fastest in WK treatments, with all products reaching the goal pH of less than 4.6 within three days. In contrast, the SK treatments did not achieve a pH of 4.6 until day 14. Titratable acidity was significantly correlated with pH values and was also significantly different between species. Furthermore, all treatments had high ($\approx 10^6$ CFU/g) lactic acid bacteria populations, showing the potential of this product as a probiotic. The use of the starter cultures, *Lactobacillus plantarum* and *Leuconostoc mesenteroides,* was likely responsible for the successful fermentation of the seaweed to produce sauerkraut. However, the fermentative success of several different species of lactic acid bacteria could be studied to result in a quicker fermentation time and increased shear firmness in the SK treatments, as LAB influenced these variables. Also, the addition of more than two species of LAB could be useful in improving the fermentation success and texture. While the inoculation level was based on typical LAB populations found in sauerkraut, it could have been increased to possibly increase the probiotic benefits of the seaweed sauerkraut. Furthermore, there are some LAB that are particularly known for their probiotic benefits such as *Lactobacillus acidophilus,* that could be further investigated. Typical to most fermentations, the lactic acid concentration increased over time with the highest concentration found on day 14,

while low concentrations of acetic acid were found. As the seaweed concentration in the sauerkraut treatments increased, sugar concentrations in the brine decreased. Glucose and fructose were present in higher amounts in the brine compared to sucrose concentrations, which were negligible throughout the study. Although measurement of lactic acid and sugars was important for characterizing the extent of fermentation, quantifying additional carbohydrates may have provided more insight. Seaweeds contain a variety of polysaccharides, and it would be useful to analyze those carbohydrates and their potential derivatives to better understand how they are utilized during fermentation.

Pathogens and coliforms were detected in the sauerkraut, despite seaweeds' reported antimicrobial activity. The SK treatments had higher levels of coliforms and *Vibrio* sp. was detected in the 75% SK treatment, making *Vibrio* sp. the only pathogen confirmed out of the four (*Vibrio, Salmonella, Staphylococcus aureus, Listeria*) that were tested. Despite these results, contamination by *Vibrio* may possibly be avoided in the future, as the time and location of harvest could have effected these results. The presence of *Vibrio* sp. may have been because the seaweed was harvested at the end of May, towards the end of the harvesting season. *Vibrio* is typically more prominent in the summer/warmer months, although there is a concern that *Vibrio* levels might increase in the Gulf of Maine due to the changing climate. Overall, the results indicate that the main safety concern in the seaweed sauerkraut could be the coliforms and possibly the presence of *E. coli*. We recommend that the industry collects seaweed earlier in the season whenever possible and monitors the water quality of growing/harvesting sites. If seaweed becomes contaminated with pathogens while making seaweed sauerkraut, fermentation is the only step to kill the pathogens. This would not be a reliable method of pathogen control since not

all pathogens are killed by acidic environments. Additionally, concerns about biogenic amines in fermented products have arisen and their evaluation may be another aim for future studies.

While physicochemical and microbial differences were found in the different seaweed sauerkrauts, consumers did not rate the samples differently for acceptability of color, aroma, overall liking, flavor, saltiness, and texture. The mean scores for overall liking of all the seaweed sauerkraut samples were just under "Like slightly." To improve these scores, more testing could be done on the sauerkraut formulation, which was a very basic recipe. Garlic, caraway seed, other spices, or other ingredients could be added, which may increase consumer acceptability of the product. Furthermore, it would have been interesting to ask consumers whether they typically consumed sauerkraut plain or as a condiment. Additionally, many panelists who tried the seaweed sauerkraut did not consume sauerkraut frequently. The samples may have been rated as more acceptable if more frequent sauerkraut consumers had evaluated the products.

Seaweed sauerkraut could offer important health benefits to consumers, helping to create a niche market for this product. In addition to probiotic benefits, seaweed sauerkraut is rich in antioxidants. Although there were no differences in DPPH and TPC of SK vs WK treatments, increasing the seaweed concentrations improved TPC, FRAP values, and radical scavenging activity in the sauerkraut. Furthermore, seaweed sauerkraut may offer consumers more nutrients than dried seaweed. The heat treatment required to dry seaweed can decrease the nutrients in the products. In contrast, seaweed sauerkraut is produced using a non-thermal preservation process that could be a lower cost alternative to creating shelf stable products.

In conclusion, the different concentrations of seaweed and species of seaweed influenced the fermentation success of the product. The WK treatments seemed to be the better fermentative

substrate. WK had shear firmness values similar to cabbage and the product was crisp and crunchy. WK also fermented quicker. In regard to concentrations, the 75% SK treatment was the least successful of the six treatments, as it took the longest to ferment, had high coliform counts, and *Vibrio* was detected. Overall, the 25% and 50% treatments seemed to be the most consistent throughout the study with each concentration having important strengths. For example, the 25% treatments fermented more quickly, while 50% treatments mostly had higher antioxidant capacities.

While the common variables that were tested throughout fermentation and storage provided important information on the fermentation dynamic, further variables could be tested to improve quality, safety, or shelf life. Extending the shelf life of the sauerkraut may be useful for commercial sales. This study lasted 60 days and the product remained stable during this time and showed no signs of spoilage. These results indicate that the shelf life of fresh seaweed was extended considerably, as the shelf life of fresh seaweeds is typically less than one week.

The recent rise in consumption of seaweeds, probiotics, and fermented products suggests that this product could be well received by consumers, and local companies believe there is market potential for seaweed sauerkraut in Maine. This study evaluated the potential for creating seaweed sauerkraut and assessed the shelf life and safety of this novel product. The results obtained offer important information to help the seaweed industry to create value-added products and diversify markets.

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APPENDICES

Appendix A: Recruitment Notice

SEAWEED SAUERKRAUT TASTING

Are you interested in trying seaweed sauerkraut?

If you are at least 18 years old and like eating sauerkraut, please help evaluate seaweed sauerkraut as part of MS thesis research at the University of Maine.

Testing will take about 15 minutes to complete. Participants will be provided with 3 dollars cash for tasting the seaweed sauerkraut and completing a survey.

Testing will be held on: March 27, 2018.

Testing will take place between the hours 11:00am-3:00pm at the Sensory Evaluation Center located in Hitchner hall (Room 158A and 158B).

Please e-mail sarah.brochu@maine.edu for more information.

If you do not like eating sauerkraut or seaweed, or have an allergy to seafood, we ask that you please do not participate.

Appendix B: Seaweed Sauerkraut Informed Consent

Dear Seaweed Sauerkraut Consumer,

You are invited to take part in a Master's thesis project titled "Development and Evaluation of a Novel Fermented Sea Vegetable Product Using Commercially-important Maine Sea Vegetables" by Sarah Brochu and Denise Skonberg, in the School of Food and Agriculture at the University of Maine. The purpose of this research is to learn about consumer acceptability of seaweed sauerkraut. You must be at least 18 years of age to take part in this survey.
Please do not participate if you do not like sauerkraut or seaweed or if you are allergic to seafood

What Will You Be Asked to Do?
If you decide to take part in this survey, you will be asked to answer a few questions about yourself. Then, you will be served four seaweed sauerkraut samples. It may take up to 15 minutes to answer all questions.

Risks:
The risks of this study are minimal, just the loss of your time and inconvenience. Risks are no greater than those associated with typical eating.

Benefits:
You may enjoy eating the different seaweed sauerkraut samples. The overall potential benefit of the research is the development of seaweed products with health benefits for consumers.

Compensation:
Upon completion of today's test, you will receive 3 dollars for your completion of the survey. No compensation will be provided if you decide not to complete the test.

Confidentiality:
Your name will not be on any files that contain your answers to our questions. Data will be kept in the Sensory Evaluation Center's locked office. All data will be destroyed by June 2019 or after the research is published, whichever comes first.

Voluntary
Participation is voluntary. If you choose to take part in this study, you may stop at any time, however compensation may not be granted. You may skip any questions you do not wish to answer.

Contact Information
If you have any questions about this study, please contact Sarah Brochu at (802) 917-3028 or sarah.brochu@maine.edu for more information. You may also reach the faculty advisor of this study at Denise.Skonberg@umit.maine.edu. If you have any questions about your rights as a research participant, please contact Gayle Jones, Assistant to the University of Maine's Protection of Human Subjects Review Board, at 581-1498 (or e-mail gayle.jones@umit.maine.edu).

Appendix C: Seaweed Sauerkraut Questionnaire

Thank you for taking the time to participate in our research.

Please indicate your gender:
o Male
o Female
o Would prefer not to say

Please indicate your age range:
o 18-24
o 25-31
o 32-38
o 39-45
o 46-52
o 53-59
o 60+

About how often do you consume sauerkraut?
o Less than once per year
o 1-4 times per year
o 1-2 times per month
o 1-2 times per week
o 3+ times per week

About how often do you consume seaweed?
o Less than once per year
o 1-4 times per year
o 1-2 times per month
o 1-2 times per week
o 3+ times per week

About how often do you eat foods containing probiotics? Probiotics are live bacteria that provide health benefits when consumed.
o Less than once per year
o 1-4 times per year
o 1-2 times per month
o 1-2 times per week
o 3+ times per week

Did you know that fermented foods, such as sauerkraut, contain probiotics that are associated with disease prevention and improved digestion?
o Yes
o No

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 color of this sample?
o Dislike extremely
o Dislike very much
o Dislike moderately
o Dislike slightly
o Neither like nor dislike
o Like slightly
o Like moderately
o Like very much
o Like extremely

How much do you like the aroma of this sample?
o Dislike extremely
o Dislike very much
o Dislike moderately
o Dislike slightly
o Neither like nor dislike
o Like slightly
o Like moderately
o Like very much
o Like extremely

How much do you like the flavor of this sample?
o Dislike extremely
o Dislike very much
o Dislike moderately
o Dislike slightly
o Neither like nor dislike
o Like slightly
o Like moderately
o Like very much
o Like extremely

How much do you like the texture of this sample?
o Dislike extremely
o Dislike very much
o Dislike moderately
o Dislike slightly
o Neither like nor dislike
o Like slightly
o Like moderately
o Like very much
o Like extremely

Please rate the intensity of saltiness
o Not nearly salty enough
o Not salty enough
o Just about right
o Too salty
o Much too salty

Please rate the intensity of sourness:
o Not nearly sour enough
o Not sour enough
o Just about right
o Too sour
o Much too sour

How much do you like this sample overall?
o Dislike extremely
o Dislike very much
o Dislike moderately
o Dislike slightly
o Neither like nor dislike
o Like slightly
o Like moderately
o Like very much
o Like extremely

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

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.

BIOGRAPHY OF THE AUTHOR

Sarah Brochu was born in Hardwick, Vermont on October 3, 1993. She was raised in Hardwick, Vermont and graduated Hazen Union High School in 2012. She attended Maine Maritime Academy and graduated in 2016 with a Bachelor's degree in Marine Biology and a minor in International Business and Logistics. She entered the Food Science and Human Nutrition graduate program at the University of Maine in 2016. After receiving her degree, Sarah will start working in industry in August as a Quality Assurance Manager at Hancock Gourmet Lobster company. Sarah is a candidate for the Master of Science degree in Food Science and Human Nutrition from the University of Maine in August 2018.