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The Effect Of Post-Harvest Storage Temperature And Drying Method On The Pathogen Load Of Edible Kelp

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THE EFFECT OF POST-HARVEST STORAGE TEMPERATURE AND DRYING METHOD ON THE PATHOGEN LOAD OF EDIBLE KELP

BY

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This thesis has been examined and approved.

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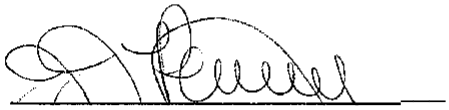

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DEDICATION

This thesis is dedicated to Connor Alexander Williams, a close friend and inspiring physicist. Without his tutorship in physics, I would not have been accepted into the Marine Sciences program at the University of New England. I am forever grateful.

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ABSTRACT

THE EFFECT OF POST-HARVEST STORAGE TEMPERATURE AND DRYING METHOD ON THE PATHOGEN LOAD OF EDIBLE KELP

by

Jessica Vorse

University of New England, August, 2022

The American seaweed industry is growing, primarily into the edible sector, and as a result, more seaweed products are available for human consumption. It is necessary to evaluate the safety of industry's current post-harvest storage and processing methods to ensure the risk of foodborne pathogens on edible seaweed remains low. We evaluated the pathogen load of edible kelp post-harvest under three different storage temperatures (4˚C, 10˚C, 20˚C) and two different drying methods (air- and freeze-drying). The focal pathogens for this project were six of the most common food-associated pathogens in the United States: *Listeria monocytogenes, Salmonella enterica, Staphylococcus aureus,* pathogenic *Escherichia coli, Vibrio vulnificus* and *Vibrio parahaemolyticus*. We tested all six pathogens under each treatment condition on both sugar kelp (*Saccharina latissima)* and rockweed (*Ascophyllum nodosum*) as these are the most commonly farmed and wild-harvested species in Maine, respectively. We inoculated a known concentration of pathogen onto freshly harvested kelp, treated it under a storage temperature or drying method, and sampled it over time to determine the impact of treatment on pathogen load. Our results showed that storage at 20˚C led to replication while storage at 4˚C and 10˚C were effective at halting the replication of focal pathogens; however, as expected, no storage temperature resulted in notable pathogen death. On the contrary, both air-drying and freeze-drying were effective means to produce significant log scale reductions in

surface pathogen load for all focal species. For both kelp species and a majority of pathogens, air-drying reduced pathogen load more than freeze-drying. Lastly, storing dried kelp for 6-weeks further reduced pathogen load across all cases. These results are promising for industry as they corroborate historical evidence that current post-harvest storage and processing conditions are producing products safe for human consumption.

INTRODUCTION

Maine Edible Seaweed Industry

The United States is in the midst of shaping a rapidly-growing domestic edible seaweed industry. The latest estimates report that the U.S. produces 385,000-431,000 wet kg of edible seaweed annually, with Maine producing the majority of this supply for both wild harvested and farmed sectors (Piconi et al., 2020). Almost the entire US industry of edible seaweed is comprised of a single species – sugar kelp (*Saccharina latissima*). From 2015 to 2020 the wet pounds of Maine farmed seaweed have increased by over 30-fold with a total harvest of 225,502 wet kg of sugar kelp, valued at $301,285.60 for the 2020 season (Piconi et al., 2020; Maine DMR, 2020). It is projected that by 2035 Maine farmed edible seaweed landings will increase to 1.4 million wet kg (Piconi et al., 2020). That will bring a significant amount of money into the state considering that in 2019, Maine's edible seaweed sector contributed $13.4 million to the state economy (Piconi et al., 2020). Not included in these estimates is another important species in Maine's seaweed economy - rockweed (*Ascophyllum nodosum*). Though rockweed is consumed directly in teas and as ingredients in other specialty food items, it is not often considered an edible seaweed. Rockweed is Maine's most commonly wild harvested kelp species and has had landings increase from 2.2 million kg in 2001 to 6.7 million kg in 2019, with harvest valued at $590,927 (Maine DMR, 2021). Since edible seaweed harvest and farming requires seasonal, part-time commitment, many industry players use it as an income supplement. Considering that the majority of edible seaweed workers are involved in other sectors of Maine's seafood industry, the edible seaweed sector serves

the important function of diversifying and, thus, protecting the economic stability of Maine's coastal economy (Piconi et al., 2020).

Food Safety Regulations and Edible Seaweed

The projected growth of Maine's edible seaweed sector will result in increased accessibility of edible seaweed products in direct consumption channels (Piconi et al., 2020). While the public will be increasingly exposed to seaweed as a food product, edible seaweed has yet to be regulated by the FDA. In 2011, the FDA Food Safety Modernization Act (FSMA) was signed into law. This law expanded the regulatory authority of the FDA, allowing them to increase their focus on foodborne illness prevention and not just outbreak response. Through legislative mandate the FSMA requires that science-based preventative controls are in place across the entire food supply chain to mitigate the risk of any potential hazard. Specifically, food facilities are required to submit a Hazard Analysis and Risk-based Preventative Controls (HARPC) plan. The HARPC has five intentions: identify potential hazards, implement validated controls to mitigate/prevent those hazards, monitor the specified controls, record all monitoring, and create a plan of response if a control fails (FDA, 2018; FSMA, 2011). The HARPC is a regulation that applies across food industries; however, there are more specific FDA regulations that individual industries, like produce and shellfish, must comply with, as well.

Produce and shellfish are the foodstuffs most closely matching seaweed's regulatory needs; however, edible seaweed is a unique good and cannot be grandfathered into either regulatory program (National Sea Grant Law Center webinar, 8/28/20). As of 2016, under the FSMA, the FDA enacted the Produce Safety rule establishing "science-

based minimum standards for the safe growing, harvesting, packing, and holding of fruits and vegetables grown for human consumption" (FDA, 2021, About the final rule). Similarly, shellfish is monitored by the FDA through the National Shellfish Sanitation Program (NSSP) and the Seafood Hazard Analysis Critical Control Point (Seafood HACCP) program which aim to identify and control for sanitation hazards in both raw and cooked shellfish from harvest through processing, shipping, and merchandising (FDA, 2020; FDA, 2022). Included in all of these regulatory programs are science-based monitoring and control procedures to mitigate bacterial pathogen risk. The edible seaweed industry is expanding so rapidly that soon industry players will need to comply with the FSMA by creating an HARPC, and possibly industry-specific regulations similar to that outlined above. Concerningly, based on the industry's science to date, it does not have the information needed for compliance due to lack of consensus regarding applicable hazards and corresponding absence of validated control measures.

In addition to a lack of federal food safety regulation, there are also no in-state (Maine) regulations for seaweed as a food product. Other select states with burgeoning edible seaweed industries are beginning to construct regulatory guidelines. In Connecticut, all aquaculture producers need to be trained on and compliant with the Seafood HACCP. This includes seaweed farmers and processors; however, as mentioned above, seaweed is not currently regulated under the Seafood HACCP. The Connecticut Sea Grant (CSG), in partnership with the Connecticut Department of Agriculture Bureau of Aquaculture (DABA), put together a Seaweed Guide to be referenced along with the FDA's Fish and Fishery Products Hazards and Controls Guidance (FDA Hazards Guide) in an attempt to specify guidance and regulation for industry. The Seaweed Guide

outlines potential hazards and associated controls related to seaweed processing and states that waters where seaweed is grown must pass the quality testing required for shellfish aquaculture. Growers need to have seaweed tested by a state or certified lab for pathogens of concern before the product can be sold for further processing or consumption. Further, the Seaweed Guide notes that seaweed should be stored at ≤ 41˚F (5˚C) within 2 hours of harvest and must not be exposed to temperatures over 40˚F (4.45˚C) for more than two hours during processing. Lastly, a water activity level of 0.85 or below is recommended for shelf stable dried products (Concepcion et al., 2020).

While the Connecticut Seaweed Guide is intended to maintain product safety, there is a limited set of published studies conducted with domestic seaweed species to corroborate that the suggested controls are the most appropriate. Since the guidelines are based on the Seafood HACCP they are reminiscent of shellfish regulation. While compliance with the Seafood HACCP is likely to be adopted for the edible seaweed industry in other states, regulating seaweed in a similar fashion to shellfish may be inappropriate given the differences between the two foodstuffs. For example, the Seaweed Guide makes multiple references to seaweed quality being closely linked to surrounding water quality when the published science to date does not support this assumption. Barberi et al. (2020) discovered that the pathogen load on freshly harvested sugar kelp was inconsistent with, and often lower than, the pathogen load of surrounding water off the coast of Southern Maine. Additionally, on a kelp farm in Long Island Sound, NY, distinct differences were found between the microorganism community on sugar kelp and in the surrounding water column. These differences were explained by sugar kelp being more selective regarding colonization by microorganisms (Liu et al.,

2022). Such findings suggest that living seaweed may modulate colonization of food pathogens on its surface; however, once the seaweed is harvested it may lose this ability. Indeed, edible seaweed is likely the most susceptible to pathogen contamination and replication during the post-harvest stage. If edible seaweed is to be regulated, it is important that the controls put in place are based on sound scientific research tailored to the product and its specific vulnerabilities.

Food Safety Research

Recent reports, while somewhat contradictory, indicate that edible seaweed is potentially subject to colonization by ocean-borne pathogens. A study conducted in Long Island Sound, NY, looking specifically for pathogenic *Vibrio* spp., identified no *Vibrio vulnificus* or *Vibrio parahaemolyticus* on sugar kelp. Some non-pathogenic *Vibrio* spp. were recovered at the end of the harvest season in May, but only on the old blade tips (Liu et al., 2022). Conversely, Barberi et al. (2020) surveyed three sugar kelp farms along Maine's southern coast and detected a very low but frequent presence of enterohemorrhagic *Escherichia coli* O157:H7, *V. parahaemolyticus*, *Vibrio alginolyticus* and *Salmonella enterica* serovar Typhimurium on freshly harvested samples.

As a means to control potential cases of pathogen contamination and preserve product after harvest, raw seaweed meant for human consumption is typically subject to temperature control via refrigeration. Alternatively, seaweed subject to secondary processing is most often air-dried for dry storage or blanched for freezer storage (Piconi et al., 2020). Several studies have examined the effect of freezing, boiling/blanching, and air-drying on the microbial load of *S. latissima* and *Alaria esculenta* (winged kelp) (Blikra et al., 2018; Akomea-Frempong et al., 2021; Lytou et al., 2021). These studies

aimed to examine the effect of processing on naturally-contaminated seaweed that was not artificially inoculated with known pathogens prior to experimentation. Although these studies provided insight on the effect of processing on microbial quality parameters, because there was little natural pathogen contamination of the seaweed, the authors were unable to elucidate the effect of the processing conditions on pathogen load. Blikra et al., (2018) analyzed a small sample of fresh and frozen *S. latissima* and *A. esculenta* grown in Norway and found no enterococci, coliforms, pathogenic Vibrios, or *Listeria monocytogenes* on any samples. Akomea-Frempong et al., (2021) sampled raw, blanched, and fermented *S. latissima* for presence of food pathogens and did not find any *Vibrio* spp. *L. monocytogenes, Salmonella* spp., or *Staphylococcus aureus*. One presumptive *Vibrio* sp. was originally detected on a raw sample but was no-longer detectable after fermentation. Lastly, Lytou et al., (2021) sampled fresh/frozen, dried, and re-hydrated seaweed from Scotland and no human pathogenic strains of *Salmonella*, *E. coli*, *S. aureus* or *L. monocytogenes* were found on *S. latissima*, but one sample of *A. esculenta* harbored *L. monocytogenes*, likely introduced after harvest.

We can conclude from the aforementioned research that food pathogens of concern associate with edible seaweeds of the North Atlantic at very low levels; however, sporadic incidences of low-level contamination have now been documented by multiple researchers. This should not be taken lightly as many of the foodborne pathogens regulated by the FDA are known to be infectious at levels ≤1,000 cells, with some strains, like enteroinvasive *E. coli* (EIEC) and *S. enterica*, having an infective dose as low as 10 cells for at-risk individuals (Schmid-Hempel & Frank, 2007). Furthermore, if edible seaweed is not properly handled post-harvest, low levels of pathogen could replicate to

high levels. In addition to public health implications, contamination of food by microbial pathogens is known to have significant economic consequences for both large and small companies, in some cases leading to the closure of a business (Hussain & Dawson, 2013). The seaweed industry needs a base of science tailored to their products for determining appropriate processing controls to protect against potential incidences of contamination.

Due to the difficulty analyzing pathogens' response to processing based on low levels of naturally-occurring contamination, we designed inoculation-based experiments to determine the effect of post-harvest storage temperature (objective 1), as well as post-harvest drying processes (objective 2), on seaweed-associated pathogen load. These processing conditions have yet to be validated in literature regarding food pathogen control for seaweed species relevant to western domestic industry. Specifically, we tested three different storage temperatures (4˚C, 10˚C and 20˚C) and two different drying methods (air- and freeze-drying). These treatments were determined to be most relevant to the industry by stakeholders during a conference held in February of 2020. Regarding objective 1, we expected pathogen replication to increase over time with increasing storage temperature. Regarding objective 2, we expected pathogen load to decrease over time, with freeze-drying resulting in larger load reductions than air-drying. Experiments were conducted with rockweed and sugar kelp, two kelp species of importance to the Maine seaweed industry. Each experiment was completed with six bacterial food pathogens - *L. monocytogenes, S. enterica, E. coli, V. parahaemolyticus, V. vulnificus* and *S. aureus.* These pathogens were chosen because they are a selection of the most common infectious food pathogens in the United States with some having specific associations with seafood products (Bintsis et al., 2017). This research will aid in the

development of state and federal food safety regulations for seaweed as a sea vegetable enabling continued industry growth and protection.

MATERIALS AND METHODS

Bacterial Strains

Multi-strain cocktails of *L. monocytogenes, S. enterica, V. vulnificus, V. parahaemolyticus, S. aureus* and *E. coli* were used for kelp inoculations (Table 1). Two to four strains of each species were combined into individual species cocktails. Stock cultures were maintained at -80˚C. Prior to experiments, cultures were subcultured onto appropriate solid growth media and isolated colonies were inoculated into liquid media and grown in a shaking incubator for 16-18 h at 37˚C (Table 1, Standard Growth Media).

In preliminary studies, select strains of *L. monocytogenes* and *V. vulnificus* (Table 1, bolded strains) exhibited poor recovery following inoculation onto kelp. To improve recovery of these pathogens we habituated these strains to a seawater/kelp environment prior to conducting kelp inoculation studies (Appendices, Habituation Protocol).

Kelp Species and Sampling Sites

All rockweed and sugar kelp samples were taken from Saco Bay, Maine, to eliminate the possibility of regional differences in the kelp introducing variability in our findings. Sugar kelp samples came from UNE's experimental farm sites (Maine DMR sites: SACO RIx and CBYR121) and were harvested from long-lines at the hold fast with a knife. Rockweed samples were wild harvested with a knife 16" above the holdfast from the intertidal zone along the Biddeford Pool coastline (Maine DMR Seaweed Harvester Reporting Sector 9-1). Harvested biomass was collected in either a clean trash bag or a mesh onion bag. Biomass was not held on ice post-harvest but was transported back to

the MSC immediately and stored in a flow-through seawater tank within 30 m of harvesting.

Kelp Sample Preparation

Rockweed and sugar kelp samples were harvested from their respective sites ≤ 3 days before experimentation and were held on-site in a flowthrough seawater tank at ambient ocean temperature. On the morning of each experiment rockweed and sugar kelp samples were cut with scissors to weigh 25 g ± 1. Rockweed samples consisted of randomly cut blade fragments. Sugar kelp samples consisted of whole 25 g segments of individual blades excluding the stipe. All biofouling organisms were removed from samples by hand.

Temperature Storage Trials

At the start of each trial, samples were inoculated with individual pathogen cocktails suspended in sterile artificial seawater (Instant Ocean Sea Salt + DI water), in which each cocktail consisted of 2-4 strains of an individual bacterial species at a total bacterial concentration of 1×10^6 cfu/g kelp. Samples were incubated with 250 mL of inoculum solution in 400 mL Whirl-Pak bags at room temperature for one hour to promote bacterial adhesion to the kelp. Following the 1h incubation, some samples (n=3) were collected immediately (0 h) to confirm association of our inoculum concentration of pathogen with the kelp. The remaining samples were moved to Ziploc bags, one 25 g sample per bag, and placed in temperature storage at either 4˚C, 10˚C or 20˚C. At 8 h, 24 h and 48 h timepoints for sugar kelp, and at 24 h and 48 h timepoints for rockweed, samples were pulled from each temperature treatment (n=3) for bacterial isolation. To

dislodge and isolate surface microbes, each 25 g kelp sample was stomached in an interscience BagMixer 400 P for 60 s in 250 mL sterile artificial seawater. The resulting bacterial suspension was diluted in DPBS and plated on selective growth media overlayed with a thin layer of standard growth media to enumerate surface pathogen load (Table 1). The thin layer overlay allowed for improved recovery of injured cells (Wu & Fung, 2001). The results are reported in colony forming units per gram of kelp (cfu/g). Temperature storage trials were performed with sugar kelp and rockweed. For each kelp species, a full trial was repeated two to four times for each of the six focal pathogens. Sugar kelp temperature storage trials were completed in Spring 2021 and rockweed temperature storage trials were completed in Fall 2022.

Drying Method Trials

To assess the effect of drying method on kelp pathogen load, kelp was inoculated with pathogen cocktails as described above. Following the 1 h incubation, some samples (n=3) were collected immediately (wet sample) to confirm association of our inoculum concentration of pathogen with the kelp. The remaining samples were dried with either air-drying or freeze-drying. Air-dry rockweed samples went into a BSL-2 rated greenhouse and were laid out on individual (Appendices, Figure 6). Air-dry sugar kelp samples went into the same BSL-2 rated greenhouse and each sample was hung by one end from a clothesline so that no samples touched (Appendices, Figure 6). Freeze-dry samples were cut with scissors from 25 g to 5 g ± 0.5, added to individual glass vials and placed in a Labconco freeze dryer for 48 hours. Once samples in both drying treatments achieved a target moisture content (28-32% for rockweed and 8-11% for sugar kelp), half underwent bacterial isolation immediately while the other half were vacuum sealed and

stored on the lab bench at room temperature undergoing bacterial isolation on their 40th day in storage (n=3). To dislodge and isolate surface microbes, the 25 g and 5 g kelp samples were stomached in 250 mL and 50 mL of artificial seawater, respectively. Dilution and plating of each sample was performed as previously described. The drying method trials were completed with sugar kelp and rockweed. For each species of kelp, a full trial was repeated two to four times for each of the six focal pathogens. Rockweed drying method trials were completed in Summer 2021 and sugar kelp drying method trials were completed in Spring 2022. Since the sugar kelp drying method trials occurred in spring when the weather was much cooler with frequent rain, to encourage drying, a Honeywell Quick Heat HZ-315 Compact Ceramic Heater and a Toshiba 70-pint 115-Volt Dehumidifier were added to the greenhouse running from ~9am - 3pm for 1 day. Both machines were adequately rated to control the temperature and humidity in our greenhouse based on its square footage.

Controls

At every sampling event, across both the storage temperature and drying method trials, one un-inoculated piece of seaweed was sampled as a control. Control samples were treated, handled, and sampled in an identical fashion to experimental samples throughout the duration of all experiments. The only difference in treatment was during the initial one hour incubation of the 25 g samples. Experimental samples were incubated in inoculum solution and control samples were incubated in sterile artificial seawater containing no addition of pathogen. During sampling, the bacterial suspension isolated from control samples was plated on the appropriate selective media for the focal pathogen being investigated. The natural contamination of each focal pathogen on control

seaweed samples was enumerated and subtracted from the amount of pathogen recovered from experimental samples. This allowed for the elimination of potential inflation of our data caused by natural pathogenic contamination of our seaweed samples.

Water Activity

In addition to evaluating the moisture content of our kelp samples on % of water weight lost, we also collected water activity (a_w) data on select samples. During every inoculation event one kelp sample was included for a_w analysis that underwent the same treatment as our control samples. All a_w samples were vacuumed sealed at the same time as the dry-storage samples in their experimental trial. The a_w samples were then set aside and kept at room temperature until all drying trials had been concluded. Water activity data was collected at the University of Maine under the guidance of Dr. Jennifer Perry using an Aqualab meter calibrated with 0.76 standard. Readings for each sample were taken in duplicate and averaged.

Focal Colony Identification

DNA sequencing was used to confirm that recovered bacteria were the same species that had been used during inoculation. Briefly, a random subsample of recovered colonies across sampling events were grown in appropriate medium (Table 1) and DNA was isolated from fresh cultures using a Qiagen DNeasy Blood and Tissue Kit. PCR was performed to amplify the 16S rRNA gene. 16S primers from Integrated Data Technologies included FOR: 5’ - AGA GTT TGA TCC TGG CTC AG and REV: 5’ - ACG GCT ACC TTG TTA CGA CTT. These primers were used for both PCR amplification and DNA sequencing. The thermal conditions for the PCR included initial

denaturation for 4 min at 94˚C, 30 cycles of 45 s at 94˚C, 45 s at 55˚C, and 1 min at 72˚C, concluded by final extension for 10 min at 72˚C (Boye et al., 1999). The resulting PCR products were purified using the DNeasy PowerClean Pro Cleanup Kit. Sanger sequencing was performed by the Yale University Keck DNA Sequencing Facility. Resulting sequences were analyzed using the NCBI BLAST standard nucleotide tool (blast.ncbi.nlm.nih.gov). Colony ID analysis does not appear in this manuscript as we have not yet received all of our samples back from sequencing.

Statistical Analysis

The limit of detection (LOD) for our plating method was 100 bacterial cells, or log 2 cfu/g kelp. Therefore, to be conservative when performing statistical analyses, in instances where pathogen was un-recoverable or recovery was below our LOD, we assigned a value of log 2 cfu/g prior to conducting the analysis.

Temperature Storage Trials

A linear mixed effects model was run in R-Studio version 2021.09.1+372 to assess pathogen load as a function of fixed variables, time in storage (0 h, 8 h, 24 h, 48 h) and storage temperature (4˚C, 10˚C, 20˚C), and random variable, trial replicate. When significance was found, multiple pairwise comparisons were run using Tukey's HSD test (Appendices, Table 2-31).

Drying Method Trials

A linear mixed effects model was run in R-Studio to assess pathogen load as a function of fixed variables, drying method (air, freeze) and product type (wet, post-dry,

dry-storage), and random variable, trial replicate. When significance was found, multiple pairwise comparisons were run using Tukey's HSD test (Appendices, Table 32-56).

RESULTS

Temperature Storage Trials

The goal of the temperature storage trials was to evaluate the effect of three storage temperatures (4˚C, 10˚C, 20˚C) on the population of six focal pathogens on the surface of kelp. Pathogen load was evaluated at multiple timepoints over 48 hours.

Sugar Kelp

S. enterica. Temperature significantly impacted *S. enterica* population on sugar kelp (Table 2-4; Figure 1, A). At 24 h the *S. enterica* load on kelp stored at 20˚C was >1 log higher than on kelp stored at 10˚C ($p < 0.05$). At 48 h the *S. enterica* load on kelp stored at 20˚C was >1 log higher than on kelp stored at 4˚C ($p < 0.01$) and 10˚C ($p < 0.001$). Conversely, there were no significant differences in pathogen load between samples stored at 4˚C and 10˚C at any timepoints. Additionally, there were no significant changes in pathogen load over time for any storage temperatures.

V. parahaemolyticus. Temperature and time significantly impacted *V. parahaemolyticus* population on sugar kelp (Table 5-7; Figure 1, B). The *V. parahaemolyticus* load on kelp stored at 20˚C for 24 h ($p < 0.001$) and 48 h ($p < 0.001$) was >1 log higher than on kelp sampled at 0 h. Kelp stored at 20˚C for 24 h had a *V. parahaemolyticus* load 1-2 log higher than kelp stored for 24 h at 4˚C ($p < 0.001$) and at 10˚C ($p = 0.001$). Kelp stored at 20˚C for 48 h had a *V. parahaemolyticus* load 2-3 log higher than kelp stored for 48 h at 4˚C ($p < 0.001$) and 10˚C ($p < 0.001$). Conversely, kelp stored at 10˚C for 48 h had a >1 log lower pathogen load than kelp stored at 10˚C for 0 h ($p < 0.05$), 8 h ($p < 0.01$), and 24

h ($p < 0.05$). Lastly, there were no significant differences in pathogen load between samples stored at 4˚C and 10˚C at any timepoints.

E. coli. Temperature significantly impacted *E. coli* population on sugar kelp (Table 8-10; Figure 1, C). The *E. coli* load on kelp stored at 20˚C for 48 h was ~1 log higher than on kelp stored at 20˚C for 0 h ($p < 0.001$), 8 h ($p < 0.01$), and 24 h ($p < 0.05$). Additionally, the *E.coli* load on kelp stored at 20˚C for 48 h was >1 log higher than on kelp stored for 48 h at both 4˚C ($p < 0.001$) and 10˚C ($p < 0.001$). Conversely, there were no significant differences in pathogen load between samples stored at 4˚C and 10˚C at any timepoints.

L. monocytogenes. Temperature and time significantly impacted *L. monocytogenes* population on sugar kelp (Table 11-13; Figure 1, D). However, *L. monocytogenes* on sugar kelp responded differently than the previous pathogens. The *L. monocytogenes* load on kelp stored at 20˚C for 48 h was >2 log lower than on kelp stored at 20˚C for 0 h ($p < 0.001$), 8 h ($p < 0.001$), and 24 h ($p < 0.001$). Additionally, the load on the 20˚C/48 h samples was >2 log lower than on samples stored for 48 h at both 4˚C ($p < 0.001$), and 10˚C ($p < 0.001$). There were no significant differences in pathogen load between samples stored at 4˚C and 10˚C at any timepoints.

S. aureus. Time significantly impacted *S. aureus* population on sugar kelp (Table 14-15; Figure 1, E). The response of *S. aureus* was similar to *L. monocytogenes* in that we observed pathogen death overtime, but for all temperatures. The *S. aureus* load at 0 h was significantly higher than on kelp sampled at 8 h ($p < 0.001$), 24 h ($p < 0.001$), and 48 h ($p < 0.001$), regardless of storage temperature.

V. vulnificus. Time significantly impacted *V. vulnificus* population on sugar kelp (Table 16-17; Figure 1, F). Similar to *L. monocytogenes* and *S. aureus,* the significant interactions were driven by the *V. vulnificus* load at 48 h which was significantly lower than at 0 h ($p < 0.001$), and 8 h ($p < 0.001$), regardless of storage temperature.

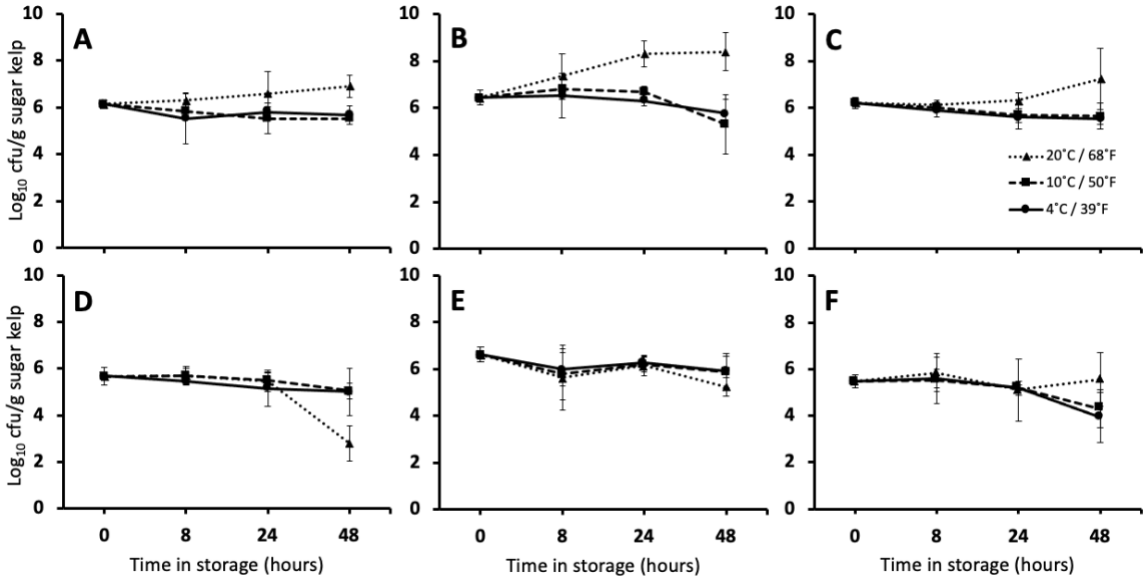

Figure 1. Effect of temperature storage on pathogen load of sugar kelp. Pathogen load was evaluated as the $\log_{10}$ colony forming units per gram of kelp, shown on the y-axis. Time kelp spent in storage (hours) is shown on the x-axis. The line patterns represent our three storage temperatures: 4˚C (solid), 10˚C (dashed), 20˚C (dotted). Each graph shows data for one of six focal pathogens: *S. enterica* (A, n = 6), *V. parahaemolyticus* (B, n = 6), *E. coli* (C, n = 6), *L. monocytogenes* (D, n = 6), *S. aureus* (E, n = 9), and *V. vulnificus* (F, n = 6) averaged across 2-3 trial replicates per pathogen. Error bars represent ± standard deviation.

Rockweed

S. enterica. Temperature and time significantly impacted *S. enterica* population on rockweed (Table 18-20; Figure 2, A). Across time we saw pathogen load decline on kelp stored at 20˚C as the load was significantly lower at both 24 h ($p < 0.05$) and 48 h ($p < 0.001$) when compared to 0 h. Additionally, kelp stored at 20˚C for 48 h had a significantly lower *S. enterica* load than kelp stored at both 4˚C ($p < 0.001$) and 10˚C ($p <$

0.01) for 48 h. Conversely, the pathogen load on kelp stored at 4˚C and 10˚C remained constant over time with no significant differences due to storage temperature.

V. parahaemolyticus. There was no significant effect of any of our three variables of interest on the *V. parahaemolyticus* load on rockweed (Table 21; Figure 2, B). Pathogen load remained constant across time and between storage temperatures.

E. coli. Time significantly impacted *E. coli* population on rockweed (Table 22-23; Figure 2, C). This significance was explained by an overall decrease in pathogen load across time, regardless of storage temperature. The *E. coli* load on kelp at both 24 h ($p < 0.01$) and 48 h ($p < 0.01$) was significantly lower than at 0 h.

L. monocytogenes. Time significantly impacted *L. monocytogenes* population on rockweed ($F_{(2, 96)} = 3.3444$, $p < 0.05$); however, multiple pairwise comparisons revealed no significant interactions between any of the sampling timepoints (Table 24-25; Figure 2, D). Additionally, there were no significant differences in pathogen load between any of the storage temperatures.

S. aureus. Temperature and time significantly impacted *S. aureus* population on rockweed (Table 26-28; Figure 2, E). The significance was explained by an overall decrease in pathogen load over time across all temperatures with the 20˚C storage treatment resulting in significantly lower pathogen load. The *S. aureus* load on kelp at both 24 h ($p < 0.001$) and 48 h ($p < 0.001$) was significantly lower than at 0 h, regardless of storage temperature. Additionally, the *S. aureus* load on kelp stored at 20˚C was

significantly lower than on kelp stored at both 4˚C ($p < 0.01$) and 10˚C ($p < 0.01$), regardless of sampling time.

V. vulnificus. Temperature and time significantly impacted *V. vulnificus* population on rockweed (Table 29-31; Figure 2, F). Uniquely, we saw pathogen load significantly decline across time on kelp stored at 4˚C as the load was ≤1 log lower at 48 h when compared to 0 h ($p < 0.001$) and 24 h ($p < 0.001$). Additionally, kelp stored at 4˚C for 48 h had a significantly lower *V. vulnificus* load than kelp stored at both 10˚C ($p < 0.01$) and 20˚C ($p < 0.001$) for 48 h. Conversely, for samples stored at 10˚C and 20˚C there were no significant differences in pathogen load across time or between samples.

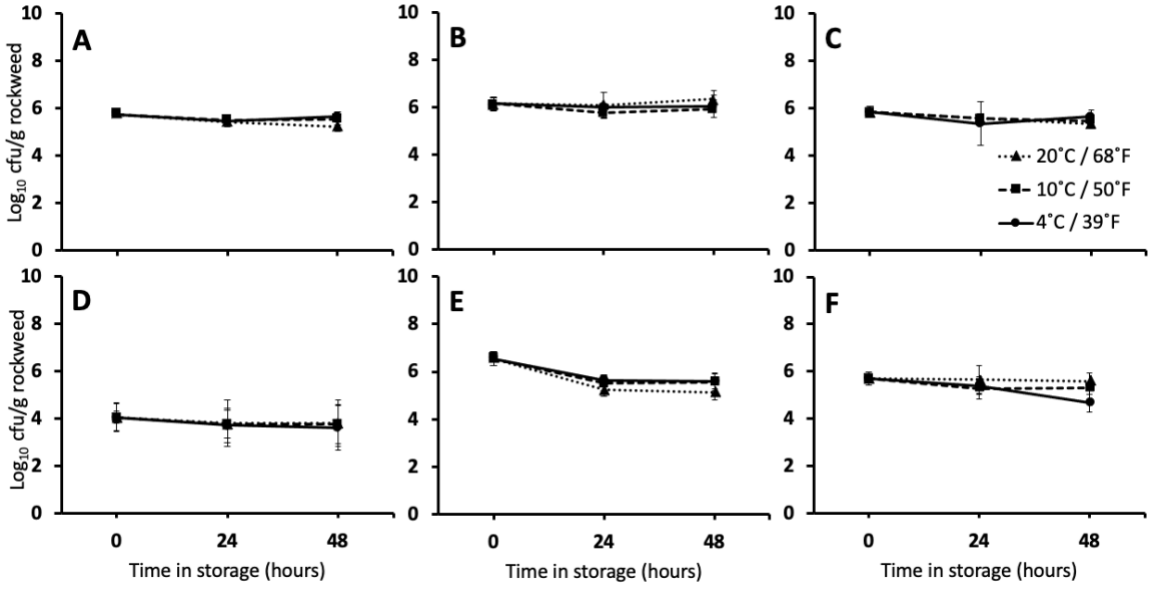

Figure 2. Effect of temperature storage on pathogen load of rockweed.
Pathogen load was evaluated as the $\log_{10}$ colony forming units per gram of kelp, shown on the y-axis. Time kelp spent in storage (hours) is shown on the x-axis. The line patterns represent our three storage temperatures: 4˚C (solid), 10˚C (dashed), 20˚C (dotted). Each graph shows data for one of six focal pathogens: *S. enterica* (A, n = 9), *V. parahaemolyticus* (B, n = 9), *E. coli* (C, n = 9), *L. monocytogenes* (D, n = 12), *S. aureus* (E, n = 9), and *V. vulnificus* (F, n = 9) averaged across 3-4 trial replicates per pathogen. Error bars represent ± standard deviation.

Drying Method Trials

The goal of the drying method trials was to evaluate the effect of two drying methods (air- and freeze-drying) on the population of six focal pathogens on the surface of kelp. Pathogen load was evaluated on wet, post-dry and dry-stored samples.

Sugar Kelp

V. parahaemolyticus. Product type significantly impacted *V. parahaemolyticus* population on sugar kelp (Table 32-33; Figure 3, A). This significance was explained by an overall reduction in pathogen load as a result of drying. The *V. parahaemolyticus* load on wet kelp was >3-4 log higher than on both post-dry ($p < 0.001$) and dry-stored kelp ($p < 0.001$), regardless of drying method. Pathogen was unrecoverable from post-dry freeze-dried samples but was recovered from post-dry air-dried samples. Though there was not a significant difference between the pathogen load of post-dry kelp and dry-stored kelp due to our LOD, we saw pathogen load was further reduced due to dry storage as *V. parahaemolyticus* was un-recoverable on dry-stored samples for both drying methods.

V. vulnificus. Product type significantly impacted *V. vulnificus* population on sugar kelp (Table 34-35; Figure 3, B). This significance was explained by a reduction in pathogen load as a result of drying. The *V. vulnificus* load on wet kelp was >3 log higher than on both post-dry ($p < 0.001$) and dry-stored kelp ($p < 0.001$), regardless of drying method. There was not a significant difference between the pathogen load of post-dry kelp and dry-stored kelp because *V. vulnificus* was un-recoverable across all dried samples, regardless of drying method or product type.

L. monocytogenes. Product type significantly impacted *L. monocytogenes* population on sugar kelp (Table 36-37; Figure 3, C). This significance was explained by an overall reduction in pathogen load as a result of drying. The *L. monocytogenes* load on wet kelp was >1 log higher than on both post-dry ($p < 0.001$) and dry-stored kelp ($p < 0.001$), regardless of drying method. Pathogen was unrecoverable from post-dry air-dried samples but was recovered from post-dry freeze-dried samples. Though there was not a significant difference between the pathogen load of post-dry kelp and dry-stored kelp due to our LOD, we saw pathogen load was further reduced due to dry storage as *L. monocytogenes* was un-recoverable on dry-stored samples for both drying methods.

E. coli. Drying method and product type significantly impacted *E. coli* population on sugar kelp (Table 38-39; Figure 3, D). This significance was driven by a reduction in pathogen load as a result of drying. The *E. coli* load on wet kelp was >1-3 log higher than on post-dry kelp that was both air- ($p < 0.001$) and freeze-dried ($p < 0.001$) as well as on dry-stored kelp that was both air- ($p < 0.001$) and freeze dried ($p < 0.001$). Additionally, for freeze-dried kelp, post-dry samples had a higher pathogen load than dry-stored samples ($p < 0.001$). For both drying methods pathogen on dry-stored samples was un-recoverable. Notably, when comparing drying methods for post-dry samples, though pathogen was recovered under both treatments, air-drying was more effective at reducing *E. coli* load than freeze-drying ($p < 0.001$).

S. enterica. Product type significantly impacted *S. enterica* population on sugar kelp (Table 40-41; Figure 3, E). This significance was explained by an overall reduction in pathogen load as a result of drying. The *S. enterica* load on wet kelp was >2-3 log higher

than on both post-dry ($p < 0.001$) and dry-stored kelp ($p < 0.001$), regardless of drying method. Pathogen was recoverable from both post-dry treatment groups. Though there was not a significant difference between the pathogen load of post-dry kelp and dry-stored kelp due to our LOD, we saw pathogen load was further reduced due to dry storage as *S. enterica* was un-recoverable on dry-stored samples for both drying methods.

S. aureus. Drying method and product type significantly impacted *S. aureus* population on sugar kelp (Table 42-43; Figure 3, F). This significance was driven by a reduction in pathogen load as a result of drying. The *S. aureus* load on wet kelp was >2-3 log higher than on post-dry kelp that was both air- ($p < 0.001$) and freeze-dried ($p < 0.001$) as well as on dry-stored kelp that was both air- ($p < 0.001$) and freeze dried ($p < 0.001$). Additionally, for freeze-dried kelp, post-dry samples had a >1 log higher pathogen load than dry-stored samples ($p < 0.001$). *S. aureus* was recoverable across all dried samples for both drying methods. Like *E. coli*, when comparing drying methods for post-dry samples, air-drying was more effective at reducing *S. aureus* load than freeze-drying ($p < 0.001$).

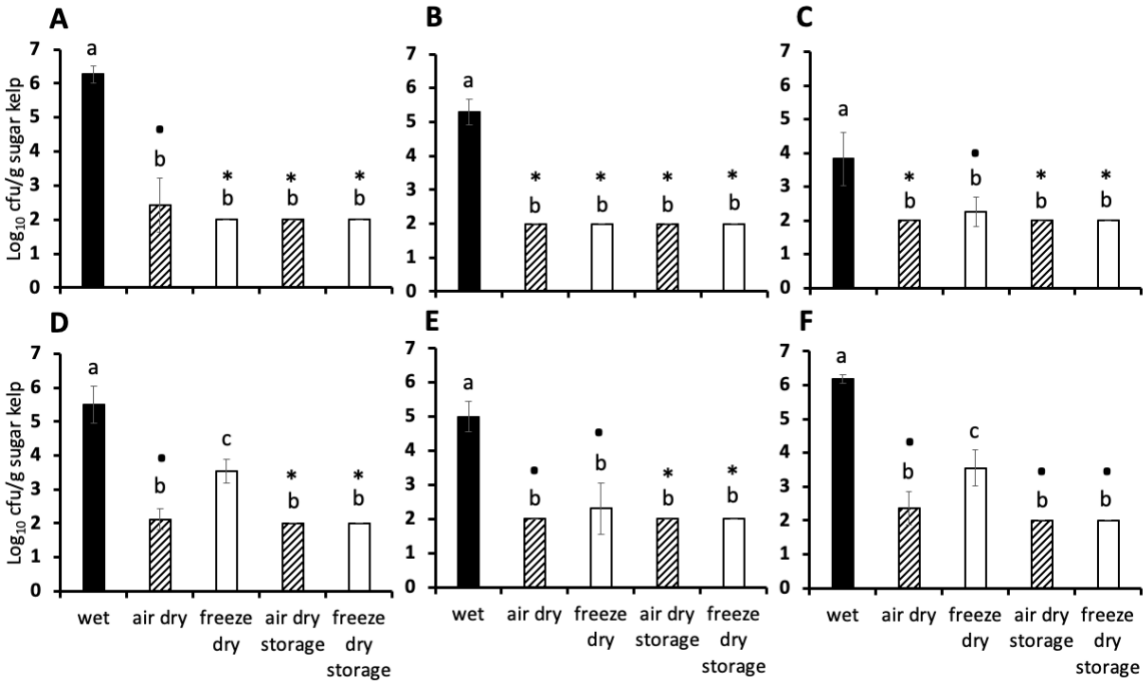

Figure 3. Effect of drying method on pathogen load of sugar kelp.
Pathogen load was evaluated as the $\log_{10}$ colony forming units per gram of kelp, shown on the y-axis. The product type sampled is shown on the x-axis: "wet" represents kelp sampled before any drying occurred, "air/freeze-dry" represents kelp sampled immediately post-dry, "air/freeze-dry storage" represents post-dry kelp that was vacuum sealed and sampled after 40 days of storage. The bar patterns represent our drying treatments: wet (black), air-dried (striped), freeze-dried (white). Each graph shows data for one of six focal pathogens: *V. parahaemolyticus* (A, n = 6), *V. vulnificus* (B, n = 6), *L. monocytogenes* (C, n = 6), *E. coli* (D, n = 6), *S. enterica* (E, n = 6), and *S. aureus* (F, n = 6) averaged across 2 replicates per pathogen. Error bars represent ± standard deviation. The a, b, and c, letters identify significant differences ($p < 0.001$) between treatments. The LOD of our sampling method was log 2 (100 cfu/g). An * represents instances of no pathogen recovery from any samples in the treatment group. An • represents instances of pathogen recovery below our LOD when pathogen was un-recoverable from some but not all replicates in a treatment group.

Rockweed

V. parahaemolyticus. Product type significantly impacted *V. parahaemolyticus* population on rockweed (Table 44-45; Figure 4, A). This significance was explained by an overall reduction in pathogen load as a result of drying. The *V. parahaemolyticus* load on wet kelp was >3 log higher than on both post-dry ($p < 0.001$) and dry-stored kelp ($p < 0.001$), regardless of drying method. Though there was not a significant difference

between the pathogen load of post-dry kelp and dry-stored kelp due to our LOD, we saw pathogen load was further reduced due to dry storage as *V. parahaemolyticus* was un-recoverable on dry-stored samples for both drying methods.

V. vulnificus. Product type significantly impacted *V. vulnificus* population on rockweed (Table 46-47; Figure 4, B). This significance was explained by an even more pronounced reduction in pathogen load as a result of drying. The *V. vulnificus* load on wet kelp was >4 log higher than on both post-dry ($p < 0.001$) and dry-stored kelp ($p < 0.001$), regardless of drying method. There was not a significant difference between the pathogen load of post-dry kelp and dry-stored kelp because *V. vulnificus* was un-recoverable across all dried samples, regardless of drying method or product type, except for one incidence of low level recovery on a post-dry air-dried sample.

L. monocytogenes. Product type significantly impacted *L. monocytogenes* population on rockweed (Table 48-49; Figure 4, C). This significance was explained by an overall reduction in pathogen load as a result of drying. The *L. monocytogenes* load on wet kelp was >2 log higher than on both post-dry ($p < 0.001$) and dry-stored kelp ($p < 0.001$), regardless of drying method. Though there was not a significant difference between the pathogen load of post-dry kelp and dry-stored kelp due to our LOD, we saw pathogen load was further reduced due to dry storage as *L. monocytogenes* was un-recoverable on dry-stored samples for both drying methods.

E. coli. Drying method and product type significantly impacted *E. coli* population on rockweed (Table 50-51; Figure 4, D). This significance was driven by a reduction in pathogen load as a result of drying. The *E. coli* load on wet kelp was >3 log higher than

on post-dry kelp that was both air- ($p < 0.001$) and freeze-dried ($p < 0.001$) as well as on dry-stored kelp that was both air- ($p < 0.001$) and freeze dried ($p < 0.001$). Additionally, for freeze-dried kelp, post-dry samples had a higher pathogen load than dry-stored samples ($p < 0.001$). For both drying methods pathogen on dry-stored samples was un-recoverable. Notably, when comparing drying methods for post-dry samples, air-drying was more effective at reducing *E. coli* load than freeze-drying ($p < 0.01$).

S. enterica. Product type significantly impacted *S. enterica* population on rockweed (Table 52-53; Figure 4, E). This significance was driven by a reduction in pathogen load as a result of drying. The *S. enterica* load on wet kelp was >2-3 log higher than on post-dry kelp that was both air- ($p < 0.001$) and freeze-dried ($p < 0.001$) as well as on dry-stored kelp that was both air- ($p < 0.001$) and freeze dried ($p < 0.001$). Additionally, for freeze-dried kelp, post-dry samples had a higher pathogen load than dry-stored samples from which we could not recover *S. enterica* ($p < 0.001$). Similar to *E. coli*, when comparing drying methods for post-dry samples, air-drying was more effective at reducing *S. enterica* load than freeze-drying ($p < 0.05$).

S. aureus. Drying method and product type significantly impacted *S. aureus* population on rockweed (Table 54-55; Figure 4, F). This significance was driven by a reduction in pathogen load as a result of drying. The *S. aureus* load on wet kelp was >1-4 log higher than on post-dry kelp that was both air- ($p < 0.001$) and freeze-dried ($p < 0.001$) as well as on dry-stored kelp that was both air- ($p < 0.001$) and freeze dried ($p < 0.001$). Additionally, for freeze-dried kelp, post-dry samples had a >2 log higher pathogen load than dry-stored samples ($p < 0.001$). *S. aureus* was recoverable across all dried samples

for both drying methods. Like *E. coli*, and *S. enterica*, when comparing drying methods for post-dry samples, air-drying was more effective at reducing *S. aureus* load than freeze-drying ($p < 0.001$).

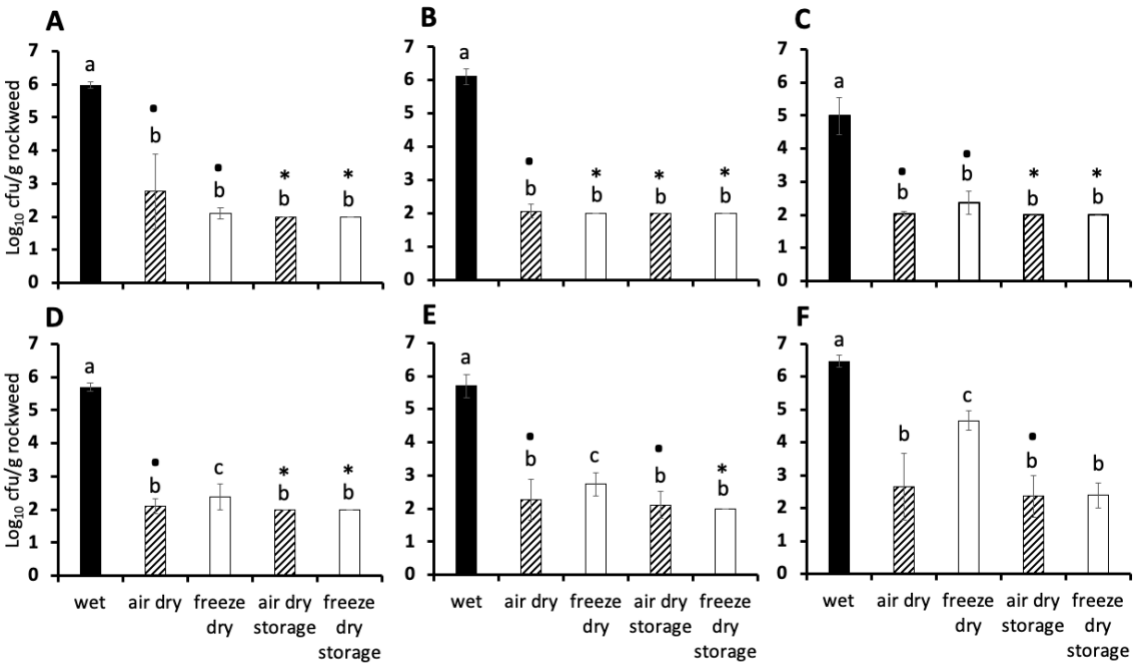

Figure 4. Effect of drying method on pathogen load of rockweed.
Pathogen load was evaluated as the $\log_{10}$ colony forming units per gram of kelp, shown on the y-axis. The product type sampled is shown on the x-axis: "wet" represents kelp sampled before any drying occurred, "air/freeze-dry" represents kelp sampled immediately post-dry, "air/freeze-dry storage" represents post-dry kelp that was vacuum sealed and sampled after 40 days of storage. The bar patterns represent our drying treatments: wet (black), air-dried (striped), freeze-dried (white). Each graph shows data for one of six focal pathogens: *V. parahaemolyticus* (A, n = 6), *V. vulnificus* (B, n = 12), *L. monocytogenes* (C, n = 6), *E. coli* (D, n = 12), *S. enterica* (E, n = 12), and *S. aureus* (F, n = 6) averaged across 2-4 trial replicates per pathogen. Error bars represent ± standard deviation. The a, b, and c, letters identify significant differences ($p < 0.05$) between treatments. The LOD of our sampling method was log 2 (100 cfu/g). An * represents instances of no pathogen recovery from any samples in the treatment group. An • represents instances of pathogen recovery below our LOD when pathogen was un-recoverable from some but not all replicates in a treatment group.

Water Activity

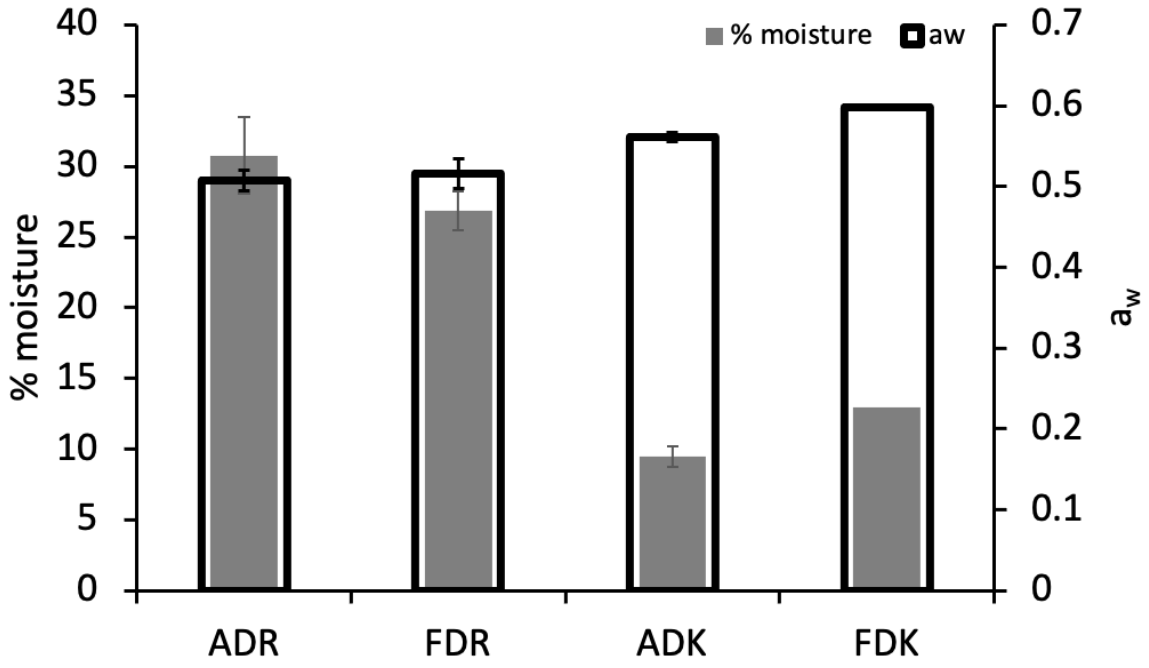

Figure 5. Moisture content and a_w readings of air- and freeze-dried rockweed and sugar kelp.
The left y-axis represents % moisture content and the right y-axis represents water activity (a_w). The x-axis represents the mean value from all samples in a treatment group: air-dried rockweed (ADR, n = 8), freeze-dried rockweed (FDR, n = 8), air-dried sugar kelp (ADK, n = 2), and freeze-dried sugar kelp (FDK, n = 1). Samples were either air- or freeze-dried following the procedures explained in the methods. The % moisture was calculated as dry weight divided by wet weight multiplied by 100. The a_w values were taken in duplicate then averaged for each sample. Error bars represent ± standard deviation.

DISCUSSION

Regarding the food safety of edible seaweed, we found that storing kelp at 4˚C was adequate to prevent pathogen replication. Additionally, drying kelp significantly reduced surface pathogen load with air-drying being more effective than freeze-drying. Further inoculation based studies are needed to determine the safest and most appropriate processing methods to maintain edible seaweed's microbial safety.

Temperature Storage Trials

Pathogens can replicate on kelp that is not subject to temperature control. Notably, *S. enterica*, *V. parahaemolyticus* and *E. coli* load increased significantly over time on kelp samples stored at 20˚C with replication occurring after just 8 hours of storage. This finding was expected as all six focal pathogens in this study can grow in temperature ranges of -0.4-10˚C (min) to 42.6-50˚C (max) and can survive under even broader ranges (Løvdal et al., 2021). This temperature tolerance also explains why we observed little pathogen death during storage at any of the tested temperatures.

We observed an unexpected reduction in *L .monocytogenes* on the surface of sugar kelp stored at 20°C for 48 hours. Considering that *L. monocytogenes*' lower limit of growth is -0.4˚C, 5.4 - 10.4 ˚C lower than the other pathogens in this study, temperature preference could be a factor explaining the population decline observed at our highest test temperature (Løvdal et al., 2021). It is also likely that *L. monocytogenes* has a sensitivity to something in sugar kelp that was released during its degradation at room temperature, leading to *L. monocytogenes* decline (Cox et al., 2010). During preliminary experiments, we observed that our lab strains of *L. monocytogenes* were virtually un-recoverable from

kelp. This sensitivity of select lab strains to kelp led us to develop our habituation protocol. Macroalgae are a known source of novel antimicrobial compounds with applications in food preservation, medicine, cosmetics, and anti-fouling solutions (Deveau et al., 2016; Silva et al., 2020; Cusson et al., 2021). This line of research has been underway for decades. In 1974 Hornsey and Hide tested 151 species of British marine algae against various bacteria of concern. They found that in the winter and spring *S. latissima* inhibited the growth of both *E. coli* and *S. aureus*. More recently, methanolic extracts from *S. latissima* were found to have over 90% growth inhibition of pathogenic strains of *L. monocytogenes* and *Salmonella abony* and spoilage strains of *Escheria faecalis* (Cox et al., 2010). Antimicrobial activity likely explains the observed decline of *L. monocytogenes* on sugar kelp stored at room temperature and could explain our other observations of pathogen load decline over time.

On sugar kelp, in addition to *L. monocytogenes*, *V. parahaemolyticus*, *S. aureus*, and *V. vulnificus* showed significant reductions in pathogen load for one or all storage temperatures. On rockweed, *S. enterica*, *E. coli*, *S. aureus*, and *V. vulnificus* all showed significant reductions in pathogen load for one or all storage temperatures. In addition to documented antimicrobial activity of sugar kelp, laminarin extracted from *A. nodosum* effectively inhibited the growth of *S. aureus*, *L. monocytogenes*, *E. coli* and *S. enterica* Typhimurium (Kadam et al., 2015). It is important to note that the studies referenced above tested kelp's antimicrobial activity against lab-reared bacterial strains. Populations of *Vibrio* spp., *E. coli*, and *S. enterica*, native to the marine environments, may be acclimated to various marine anti-microbials and less susceptible to kelp's antimicrobial action. Similarly, pathogens like *L. monocytogenes* and *S. aureus*, that establish

population in food processing facilities and on human skin, respectively, are likely to have increased exposure to anti-microbial disinfectants potentially increasing their resistance to seaweed's anti-microbials in a contamination event.

Additional factors may have contributed to the observed decline in seaweed-associated foodborne pathogens over time. Seaweed is known to have its own active and complex natural microbiome. During a comprehensive survey of associated culturable microbes, rockweed was found to have 36 genera present on its surface (Martin et al., 2015). A study of the bacterial community colonizing sugar kelp, using culture-independent sequencing methods, found 130-270 bacterial amplicon sequence variants on multiple samples (Tourneroche et al., 2020). These complex microbiomes change over time during storage. Picon et al., (2021) found that long-term refrigeration of untreated samples of edible red, green, and brown seaweeds led to decreased bacterial species richness and diversity. As seaweed is stored, successional events begin to occur on its surface resulting in certain species beginning to dominate. It is possible our pathogens were beginning to be outcompeted by other microbes. To determine how various food pathogens fit in to these complex community dynamics more research is needed.

Reports from other labs indicate that each species of seaweed has a markedly unique and distinctive microbiome (Lachnit et al., 2009; Picon et al., 2021). In our experiments with rockweed, we did not observe the same significant pathogen replication that we observed on sugar kelp. Additionally, the pathogens that showed significant population declines on rockweed were not always the same as on sugar kelp. This could be due to microbiome differences in rockweed and sugar kelp, which could differentially impact the success of pathogens on their surface. This nuance implies that each species of

edible seaweed may have different pathogens of concern and could need varying food safety regulations.

The differences in pathogen behavior between our kelp species could also be impacted by the way the different habitats of rockweed and sugar kelp have shaped their physiology. Sugar kelp grows in deeper waters where it remains constantly submerged. As a result, when harvested it breaks down rapidly. This rapid decay could create an unstable environment where pathogens are able to replicate or could become unfavorable leading to pathogen decline. Conversely, rockweed grows in the intertidal zone and is regularly exposed to oxygen, heat, and light at every low tide. Rockweed does not degrade as rapidly as sugar kelp; and therefore, appears to offer a more consistent environment for the pathogens colonizing its surface. As a result, pathogen load on rockweed trended to be more stable than on sugar kelp.

Drying Method Trials

Drying significantly reduced viability of all pathogens on the surface of kelp. This reduction became even more pronounced for all treatments after dried kelp samples had been vacuum sealed and stored for 6 weeks. Drying, defined as the removal of available water, is a popular preservation technique for a wide variety of foods because without enough available water, microbes, particularly food pathogens, cannot replicate. The amount of water available for biological processes in organic tissue is measured as water activity (a_w). The accepted standard a_w to prevent the replication of food pathogens is $\leq$0.85 (Beuchat et al., 2011). Our dried kelp exhibited a_w from 0.49 - 0.60, which does not support growth of the pathogens used in our study (Figure 5). *S. aureus* is well-documented for its ability to tolerate lower a_w levels than other food pathogens (Beuchat

et al., 2011). In our study, although *S. aureus* did not replicate on dried kelp, it did exhibit the greatest recovery from the dried product of all pathogens tested. For this reason, lowering water activity enough to prevent bacterial replication is not, on its own, an acceptable control in food safety. A stressor should be applied to the food product to eliminate bacterial contaminants of concern and then water activity should be lowered to further ensure safety and preservation of the product.

What was promising was the magnitude of the log scale reductions we observed as a result of drying. Counter to our predictions, air-drying lead to a significantly greater reduction in pathogen load than did freeze-drying for *E. coli* and *S. aureus* on sugar kelp and for *E. coli, S. enterica,* and *S. aureus* on rockweed. Freeze-drying has frequently been documented as a poor preservation technique to improve microbial safety considering it is often used to preserve bacterial cultures in viable states (Morgan & Vesey, 2009). The effect of freeze-drying on the naturally occurring *E. coli* load of Kangkung, a semi-aquatic leafy vegetable, was explored by Shin et al., (2015). Similar to our results they found drying resulted in 2.9 log scale reduction from a natural contamination level of 6.13 log cfu/g. Bourdoux et al. (2018) investigated the effect of freeze-drying on fresh cilantro inoculated with cocktails of pathogenic *E. coli*, *S. enterica* and *L. monocytogenes* and found 1.5 - 2 log reductions for *E. coli* and *S. enterica*, respectively, and a 0.71 log reduction for *L. monocytogenes*. Though freeze-drying reduces the pathogen load on kelp's surface as effectively as on fresh produce items, the reduction from this drying process across food stuffs is not compelling enough to justify its use as a microbial control. Since air-drying is already widely used across the U.S. to dry freshly-harvested

seaweed and showed notably greater reduction in pathogen load across species, it should remain the preferred drying technique to maintain the microbial safety of edible kelp.

It is vital to re-iterate that food pathogens are robust and though low a_w can prevent replication it does not necessarily result in microbial death. When under duress, non-spore-forming pathogenic cells can enter a dormant state which allows them to survive low moisture environments for long periods of time. Pathogenic *E. coli*, *L. monocytogenes*, *Salmonella*, and *S. aureus* are known to associate with low-a_w foods and have all been documented surviving on foods with a_w lower than we achieved (< 0.49 - 0.60) for months to sometimes years (Beuchat et al., 2011; Beuchat et al., 2013). It has been well studied that the thermal resistance of food pathogens increases with decreasing a_w (Syamaladevi et al., 2016). It is also understood that freeze-drying can increase pathogens' stress tolerance (Morgan & Vesey, 2009). When a pathogenic cell is sub-lethally stressed it undergoes changes that can make it more robust to the stressful environment. These changes that can help a pathogen survive on processed food can also help a pathogen survive inside a host, increasing its virulence (Wesche et al., 2009). Again, due to limitations in the sensitivity of detection of our testing method, we cannot claim that drying processes or long-term dry storage completely eliminated pathogens from kelp's surface, but our findings do indicate that these drying processes significantly reduced pathogen populations.

Recommendations and Future Directions

Based on our findings, we recommend that fresh edible kelp be stored at or below 4˚C as soon as possible after harvest to limit growth of potential pathogenic contaminants and preserve food safety. To determine time limits for refrigerated storage, longer-term

temperature storage trials should be conducted with a more diverse array of edible seaweed species.

When considering drying techniques, air-drying outperformed freeze-drying in regard to reducing pathogen load. Additionally, storing dried product for a number of weeks increased the effectiveness of drying on pathogen load reduction. Moving forward, industry should further validate the effects of air-drying on pathogen load reduction using a sampling method more sensitive than our own.

Though drying can significantly improve the food safety of edible seaweed this study does not show sufficient evidence to classify it as a kill step (5 log reduction) for any pathogens of concern. It would be beneficial to explore the use of hurdle technology to further improve the microbial safety of edible seaweed. Hurdle technology is the application of multiple, unique stressors to a food product in rapid succession. Regarding previous discussion around the improved resistance of pathogens on dried products, pasteurization of products in the wet form is a more effective microbial control than pasteurizing dried products (Beuchat et al, 2011). Blanching wet seaweed, as a hurdle before drying, has the potential to serve as a pasteurization step and a recent study found consumers preferred the texture of blanched kelp to raw kelp in fresh kelp salad (Akomea-Frempong et al., 2021). If industry wishes to further increase the food-safety of their products while maintaining sensory quality, the effects of blanching before air-drying should be explored.

Before formal kill steps can be validated and implemented for a food product it is customary for industry to focus on one pathogen of particular concern. Our results demonstrated that pathogens behave differently on the surface of a single seaweed

species and the behavior of a specific pathogen is not consistent across seaweed species. This variability highlights the potential need to identify different pathogens of concern for each seaweed species. To enable this, more sampling of product across the harvest and processing chain is needed. One potentially pathogenic microbe that was not included in our experiments is *Bacillus* spp. (Løvdal et al., 2021). Pathogenic species of *Bacillus* have been isolated from edible species of kelp (Blikra et al., 2019; Lytou et al., 2021). This pathogen is especially robust because it forms spores that are highly resistant to acidity, heat, and dehydration and it should be considered in future studies. Once a primary pathogen of concern is identified, further inoculation based experiments will be needed to gain a deeper understanding of that pathogen's behavior on such a unique food.

REFERENCES

Akomea-Frempong, S., Skonberg, D. I., Camire, M. E., & Perry, J. J. (2021). Impact of Blanching, Freezing, and Fermentation on Physicochemical, Microbial, and Sensory Quality of Sugar Kelp (Saccharina latissima). *Foods*, *10*(10), 2258. https://doi.org/10.3390/foods10102258

Barberi, O. N., Byron, C. J., Burkholder, K. M., St. Gelais, A. T., & Williams, A. K. (2020). Assessment of bacterial pathogens on edible macroalgae in coastal waters. *Journal of Applied Phycology*, *32*(1), 683–696. https://doi.org/10.1007/s10811-019-01993-5

Beuchat, L., Komitopoulou, E., Betts, R., Beckers, H., Bourdichon, F., Joosten, H., Fanning, S., & ter Kuile, B. (2011). Persistence and survival of pathogens in dry foods and dry food processing environments. *ILSI Europe*.

Beuchat, L. R., Komitopoulou, E., Beckers, H., Betts, R. P., Bourdichon, F., Fanning, S., Joosten, H. M., & Ter Kuile, B. H. (2013). Low–Water Activity Foods: Increased Concern as Vehicles of Foodborne Pathogens. *Journal of Food Protection*, *76*(1), 150–172. https://doi.org/10.4315/0362-028X.JFP-12-211

Bintsis, T. (2017). Foodborne pathogens. *AIMS Microbiology*, *3*(3), 529–563. https://doi.org/10.3934/microbiol.2017.3.529

Blikra, M. J., Løvdal, T., Vaka, M. R., Roiha, I. S., Lunestad, B. T., Lindseth, C., & Skipnes, D. (2019). Assessment of food quality and microbial safety of brown macroalgae (Alaria esculenta and Saccharina latissima). *Journal of the Science of Food and Agriculture*, *99*(3), 1198–1206. https://doi.org/10.1002/jsfa.9289

Bourdoux, S., Rajkovic, A., De Sutter, S., Vermeulen, A., Spilimbergo, S., Zambon, A., Hofland, G., Uyttendaele, M., & Devlieghere, F. (2018). Inactivation of Salmonella, Listeria monocytogenes and Escherichia coli O157:H7 inoculated on coriander by freeze-drying and supercritical CO2 drying. *Innovative Food Science & Emerging Technologies*, *47*, 180–186. https://doi.org/10.1016/j.ifset.2018.02.007

Boye, K., Høgdall, E., Borre, M. (1999). Identification of bacteria using two degenerate 16s rRNA sequencing primers. *Microbiol Res. 154*(1), 23-6.

Concepcion, A., DeRosia-Banick, K., & Balcom, N. (2020). *Seaweed Production and Processing in Connecticut: A Guide to Understanding and Controlling Potential Food Safety Hazards*. Connecticut Sea Grant.

Cox, S., Abu-Ghannam, N., & Gupta, S. (2010). An assessment of the antioxidant and antimicrobial activity of six species of edible Irish seaweeds. *International Food Research Journal*, 205–220.

Cusson, A. J., Burkholder, K. M., Byron, C. J., Grebe, G. S., & Deveau, A. M. (2021). Impact of time of harvest and drying method on antimicrobial activity of Saccharina latissima against two Staphylococcus aureus strains. *Applied Phycology*, *2*(1), 80–88. https://doi.org/10.1080/26388081.2021.1996208

Deveau, A. m., Miller-Hope, Z., Lloyd, E., Williams, B. s., Bolduc, C., Meader, J. m., Weiss, F., & Burkholder, K. m. (2016). Antimicrobial activity of extracts from macroalgae Ulva lactuca against clinically important Staphylococci is impacted by lunar phase of macroalgae harvest. *Letters in Applied Microbiology*, *62*(5), 363–371. https://doi.org/10.1111/lam.12563

FDA Food Safety Modernization Act, 21 U.S.C & 2201 (2011). https://www.govinfo.gov/content/pkg/PLAW-111publ353/pdf/PLAW-111publ353.pdf

FDA. (2018, January 30). *Background on the FDA Food Safety Modernization Act (FSMA)*. https://www.fda.gov/food/food-safety-modernization-act-fsma/background-fda-food-safety-modernization-act-fsma

FDA. (2020, October 29). *National Shellfish Sanitation Program (NSSP)*. https://www.fda.gov/food/federalstate-food-programs/national-shellfish-sanitation-program-nssp

FDA. (2022, February 23). *Seafood HACCP*. https://www.fda.gov/food/hazard-analysis-critical-control-point-haccp/seafood-haccp

FDA. (2022, May 13). *FSMA Final Rule on Produce Safety: Standards for the growing, harvesting, packing, and holding of produce for human consumption*. https://www.fda.gov/food/food-safety-modernization-act-fsma/fsma-final-rule-produce-safety

Hornsey, I. S., & Hide, D. (1974). The production of antimicrobial compounds by British marine algae I. Antibiotic-producing marine algae. *British Phycological Journal*, *9*(4), 353–361. https://doi.org/10.1080/00071617400650421

Hussain, M. A., & Dawson, C. O. (2013). Economic Impact of Food Safety Outbreaks on Food Businesses. *Foods*, *2*(4), 585–589. https://doi.org/10.3390/foods2040585

Kadam, S. U., O'Donnell, C. P., Rai, D. K., Hossain, M. B., Burgess, C. M., Walsh, D., & Tiwari, B. K. (2015). Laminarin from Irish Brown Seaweeds Ascophyllum nodosum and Laminaria hyperborea: Ultrasound Assisted Extraction, Characterization and Bioactivity. *Marine Drugs*, *13*(7), 4270–4280. https://doi.org/10.3390/md13074270

Lachnit, T., Blümel, M., Imhoff, J. F., & Wahl, M. (2009). Specific epibacterial communities on macroalgae: Phylogeny matters more than habitat. *Aquatic Biology*, *5*(2), 181–186. https://doi.org/10.3354/ab00149

Liu, Y., Wikfors, G. H., Clark, P., Pitchford, S., Krisak, M., Dixon, M. S., & Li, Y. (2022). A deep dive into the epibiotic communities on aquacultured sugar kelp Saccharina latissima in Southern New England. *Algal Research*, *63*, 102654. https://doi.org/10.1016/j.algal.2022.102654

Løvdal, T., Lunestad, B. T., Myrmel, M., Rosnes, J. T., & Skipnes, D. (2021). Microbiological Food Safety of Seaweeds. *Foods*, *10*(11), 2719. https://doi.org/10.3390/foods10112719

Lytou, A. E., Schoina, E., Liu, Y., Michalek, K., Stanley, M. S., Panagou, E. Z., & Nychas, G.-J. E. (2021). Quality and Safety Assessment of Edible Seaweeds Alaria esculenta and Saccharina latissima Cultivated in Scotland. *Foods*, *10*(9), 2210. https://doi.org/10.3390/foods10092210

Maine DMR. (2022, February 23). *Harvest of farm-raised marine algae in Maine*. https://www.maine.gov/dmr/aquaculture/data/documents/MarineAlgaeTable2015-2020.pdf

Maine DMR. (2022, February 23). *Historical Maine rockweed landings*. https://www.maine.gov/dmr/commercial-fishing/landings/documents/rockweed.table.pdf

Martin, M., Barbeyron, T., Martin, R., Portetelle, D., Michel, G., & Vandenbol, M. (2015). The Cultivable Surface Microbiota of the Brown Alga Ascophyllum nodosum is Enriched in Macroalgal-Polysaccharide-Degrading Bacteria. *Frontiers in Microbiology*, *6*, 1487. https://doi.org/10.3389/fmicb.2015.01487

Morgan, C., & Vesey, G. (2009). Freeze-Drying of Microorganisms. In *Encyclopedia of Microbiology* (pp. 162–173). https://doi.org/10.1016/B978-012373944-5.00114-0

National Sea Grant Law Center. (2022, August 28). *Seaweed food safety: federal considerations*. Sea Grant. https://www.youtube.com/watch?v=Ap7J9dtJE4s

Picon, A., del Olmo, A., & Nuñez, M. (2021). Bacterial diversity in six species of fresh edible seaweeds submitted to high pressure processing and long-term refrigerated storage. *Food Microbiology*, *94*, 103646. https://doi.org/10.1016/j.fm.2020.103646

Piconi, P., Veidenheimer, R., & Chase, B. (2020). *Edible seaweed market analysis*. Island Institute.

Schmid-Hempel, P., & Frank, S. A. (2007). Pathogenesis, Virulence, and Infective Dose. *PLOS Pathogens*, *3*(10), e147. https://doi.org/10.1371/journal.ppat.0030147

Shin, L. E. R., Zzaman, W., Kuang, Y. T., & Bhat, R. (2015). Influence of Dehydration Techniques on Physicochemical, Antioxidant and Microbial Qualities of Ipomoea aquatica Forsk.: An Underutilized Green Leafy Vegetable. *Journal of Food Processing and Preservation*, *39*(6), 1118–1124. https://doi.org/10.1111/jfpp.12326

Silva, A., Silva, S. A., Carpena, M., Garcia-Oliveira, P., Gullón, P., Barroso, M. F., Prieto, M. A., & Simal-Gandara, J. (2020). Macroalgae as a Source of Valuable Antimicrobial Compounds: Extraction and Applications. *Antibiotics*, *9*(10), 642. https://doi.org/10.3390/antibiotics9100642

Standard Nucleotide BLAST. (n.d.). Retrieved July 28, 2022, from https://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastn&BLAST_SPEC=GeoBlast&PAGE_TYPE=BlastSearch

Syamaladevi, R. M., Tang, J., Villa-Rojas, R., Sablani, S., Carter, B., & Campbell, G. (2016). Influence of Water Activity on Thermal Resistance of Microorganisms in Low-Moisture Foods: A Review. *Comprehensive Reviews in Food Science and Food Safety*, *15*(2), 353–370. https://doi.org/10.1111/1541-4337.12190

Tourneroche, A., Lami, R., Burgaud, G., Domart-Coulon, I., Li, W., Gachon, C., Gèze, M., Boeuf, D., & Prado, S. (2020). The Bacterial and Fungal Microbiota of Saccharina latissima (Laminariales, Phaeophyceae). *Frontiers in Marine Science*, *7*. https://www.frontiersin.org/article/10.3389/fmars.2020.587566

Wesche, A. M., Gurtler, J. B., Merks, B. P., & Ryser, E. T. (2009). Stress, Sublethal Injury, Resuscitation, and Virulence of Bacterial Foodborne Pathogens†. *Journal of Food Protection*, *72*(5), 1121–1138. https://doi.org/10.4315/0362-028X-72.5.1121

Wu, V. C. H., & Fung, D. Y. C. (2001). Evaluation of Thin Agar Layer Method for Recovery of Heat-Injured Foodborne Pathogens. *Journal of Food Science*, *66*(4), 580–583. https://doi.org/10.1111/j.1365-2621.2001.tb04605.x

APPENDICES

Habituation Protocol

Prior to experiments, cultures maintained at -80˚C were subcultured onto appropriate solid growth media and isolated colonies were inoculated into liquid media and grown in a shaking incubator for 16-18 h at 37˚C (Table 1, Standard Growth Media). After incubation, the absorbance at 600nm for all liquid cultures was recorded using a spectrophotometer. A sub-culture of each original liquid culture was then made in consistent media plus 2% NaCl and grown in a shaking incubator for 16-18 h at 37˚C. After incubation a new absorbance at 600nm was taken on each culture. This process continued on subsequent days, increasing cultures to 3.5% NaCl (average salinity of ocean water) and then maintaining 3.5% salinity and adding first 0.01%, 0.05% then 0.1% seaweed homogenate. A portion of the final cultures (3.5% salinity and 0.1% seaweed) was frozen down in -80˚C stocks to be used for temperature storage and drying method trials. The remaining was used in inoculation and recovery experiments.

For inoculation and recovery experiments a 15 g sample of rockweed in 150 mL of artificial seawater was inoculated with 1 x 10^6 cfu/g of the select habituated strain, incubated at room temperature for 1 hour, stomached, and then 50 mL of the bacterial suspension was sampled. The bacterial sample was then isolated from the suspension, diluted, and plated on selective growth media overlayed with a thin layer of general growth media to enumerate surface pathogen load (Wu & Fung, 2001) (Table 1). Colonies were then counted to ensure habituated strains showed enough recovery to be used in the seaweed processing trials.

Seaweed homogenate was made fresh each day that it was added to cultures. Depending on what was available, a combination blend totaling 25 g or 25 g of either rockweed or sugar kelp was added to a blender with 250 mL artificial seawater and blended until a homogenous solution was achieved. 1 mL of the solution was pulled and diluted in 9 mL of artificial seawater. This 1:10 dilution was syringe filtered at 0.2 - 0.45 µm and then added in appropriate volumes to liquid overnight cultures.

Focal Strains

Table 1. Focal pathogens and their growth medias.
Bolded strain number indicates strain used in experiments was habituated. Liquid cultures were grown in a shaking incubator at 37˚C for 16-18 hours. Plated cultures were grown in a stationary incubator at 37˚C for 24 hours except for *L. monocytogenes* which was incubated for 48 hours.

Bacterium	**Strain Description**	**Source**	**Liquid Growth Media**	**Standard Growth Media**	**Selective Growth Media**
L. monocytogenes	**Serotype 4b**	ATCC 19115	BHI + 3.5% NaCl + 0.1% SWH	TSA + 0.6% YE	Palcam – BD
L. monocytogenes	**Serotype 1/2a**	ATCC 19111	BHI + 3.5% NaCl + 0.1% SWH	TSA + 0.6% YE	Palcam – BD
L. monocytogenes	**F4244, Serotype 4b**	Dr. Arun Bhunia, Purdue Univ. (Bailey et al., 2017)	BHI + 3.5% NaCl + 0.1% SWH	TSA + 0.6% YE	Palcam – BD
L. monocytogenes	**Strain 10403S, Serotype 1/2a**	MOR 1	BHI + 3.5% NaCl + 0.1% SWH	TSA + 0.6% YE	Palcam – BD
S. enterica Javiana	FDA CFSAN001992 (human stool isolate)	Dr. Marc Allard, FDA (Allard et al., 2013)	TSB	TSA	XLD – HIMEDIA
S. enterica Enteritidis	Almond isolate	ATCC BAA-1045	TSB	TSA	XLD – HIMEDIA
S. enterica Typhimurium	ST LT2	ATCC 700720	TSB	TSA	XLD – HIMEDIA
E. coli O26:H11	STEC strain	ATCC BAA-1653	TSB	TSA	CHROMagar O157
E. coli O111:H8	STEC strain	ATCC BAA-184	TSB	TSA	CHROMagar O157
E. coli 0127:H6	EPEC strain	BEI Resources	TSB	TSA	CHROMagar O157
E. coli 0124:NM	EIEC strain	ATCC #43893	TSB	TSA	CHROMagar O157
Vibrio parahaemolyticus	Strain EB101	ATCC 17802	Nutrient Broth + 3% NaCl	Nutrient Agar + 3% NaCl	CHROMagar Vibrio
Vibrio parahaemolyticus	Strain 2B23	Dr. Jennifer Perry, Univ. Maine	Nutrient Broth + 3% NaCl	Nutrient Agar + 3% NaCl	CHROMagar Vibrio
Vibrio parahaemolyticus	Strain 279	ATCC 33847	Nutrient Broth + 3% NaCl	Nutrient Agar + 3% NaCl	CHROMagar Vibrio
Vibrio vulnificus	1B81, Avirulent	Dr. Jennifer Perry, Univ. Maine	TSB	TSA	CHROMagar Vibrio
Vibrio vulnificus	**Strain 324, CDC B9629**	ATCC 27562	TSB + 3.5% NaCl + 0.1% SWH	TSA	CHROMagar Vibrio
S. aureus	MSSA, clinical isolate	ATCC 25923	TSB	TSA	Baird Parker Agar – Criterion
S. aureus	USA300, MRSA, clinical isolate	BEI Resources NR-46070	TSB	TSA	Baird Parker Agar – Criterion
S. aureus	COW	Dr. Jennifer Perry, Univ. Maine	TSB	TSA	Baird Parker Agar – Criterion

Photographic Air-drying References

Figure 6. Rockweed (left) and sugar kelp (right) samples in the air-dry tent. Rockweed samples were laid out on individual mesh squares suspended on a grated metal shelf. Each section of shelf contained experimental samples innoculated with the same pathogen species or control samples. Sugar kelp samples were suspended vertically from rows of nylon clothesline via clothespins. Each cloethesline row contained experimental samples inncoulated with the same pathogen species or control samples.

Complete Statistical Analysis

Table 2. ANOVA from the sugar kelp temperature storage trials with *S. enterica.*
ANOVA results from a linear mixed effects model analyzing the effect of storage temperature (Temp), time in storage (Time), and the interaction between Temp and Time on pathogen load. Significant comparisons are bolded.

	Sum Sq	Mean Sq	Num df	Den df	F value	p value
Temp	**8.7016**	**4.3508**	**2**	**70.020**	**19.0631**	**2.457e07*** **
Time	0.8849	0.2950	3	70.019	1.2924	0.283860
Temp:Time	**5.2417**	**0.8736**	**6**	**70.018**	**3.8278**	**0.002339** **

Table 3. Multiple pairwise comparisons from the sugar kelp temperature storage trials with *S. enterica.*
Multiple pairwise comparisons between all storage temperatures at each timepoint using Tukey's HSD test. Significant comparisons are bolded.

Temp:Time – Temp:Time	df	t	p
4˚C:0h - 10˚C:0h	70.0	0.000	1.0000
4˚C:0h - 20˚C:0h	70.0	0.000	1.0000
10˚C:0h - 20˚C:0h	70.0	0.000	1.0000
4˚C:8h - 10˚C:8h	70.0	-1.217	0.9858
4˚C:8h - 20˚C:8h	70.0	-2.836	0.1881
10˚C:8h - 20˚C:8h	70.0	-1.619	0.8962
4˚C:24h - 10˚C:24h	70.0	1.002	0.9972
4˚C:24h - 20˚C:24h	70.0	-2.840	0.1865
10˚C:24h - 20˚C:24h	**70.0**	**-3.841**	**0.0131**
4˚C:48h - 10˚C:48h	70.0	0.573	1.0000
4˚C:48h - 20˚C:48h	**70.1**	**-4.258**	**0.0034**
10˚C:48h - 20˚C:48h	**70.1**	**-4.804**	**0.0005**

Table 4. Multiple pairwise comparisons from the sugar kelp temperature storage trials with *S. enterica.*

Multiple pairwise comparisons between all timepoints at each storage temperature using Tukey's HSD test. Significant comparisons are bolded.

Temp:Time – Temp:Time	df	t	p
4˚C:0h - 4˚C:8h	70.0	2.607	0.2960
4˚C:0h - 4˚C:24h	70.0	1.373	0.9649
4˚C:0h - 4˚C:48h	70.0	1.967	0.7135
4˚C:8h - 4˚C:24h	70.0	-1.104	0.9936
4˚C:8h - 4˚C:48h	70.0	-0.573	1.0000
4˚C:24h - 4˚C:48h	70.0	0.531	1.0000
10˚C:0h - 10˚C:8h	70.0	1.246	0.9829
10˚C:0h - 10˚C:24h	70.0	2.493	0.3617
10˚C:0h - 10˚C:48h	70.0	2.607	0.2959
10˚C:8h - 10˚C:24h	70.0	1.115	0.9930
10˚C:8h - 10˚C:48h	70.0	1.217	0.9858
10˚C:24h - 10˚C:48h	70.0	0.102	1.0000
20˚C:0h - 20˚C:8h	70.0	-0.564	1.0000
20˚C:0h - 20˚C:24h	70.0	-1.802	0.8113
20˚C:0h - 20˚C:48h	70.1	-2.856	0.1802
20˚C:8h - 20˚C:24h	70.0	-1.108	0.9934
20˚C:8h - 20˚C:48h	70.1	-2.104	0.6214
20˚C:24h - 20˚C:48h	70.1	-1.050	0.9958

Table 5. ANOVA from the sugar kelp temperature storage trials with *V. parahaemolyticus.*

ANOVA results from a linear mixed effects model analyzing the effect of storage temperature (Temp), time in storage (Time), and the interaction between Temp and Time on pathogen load. Significant comparisons are bolded.

	Sum Sq	Mean Sq	Num df	Den df	F value	p value
Temp	**32.192**	**16.0961**	**2**	**71**	**42.4359**	**7.524e-13*****
Time	**6.216**	**2.0720**	**3**	**71**	**5.4625**	**0.001953****
Temp:Time	**24.341**	**4.0568**	**6**	**71**	**10.6952**	**1.940e-08*****

Table 6. Multiple pairwise comparisons from the sugar kelp temperature storage trials with *V. parahaemolyticus.*

Multiple pairwise comparisons between all storage temperatures at each timepoint using Tukey's HSD test. Significant comparisons are bolded.

Temp:Time – Temp:Time	df	t	p
4˚C:0h - 10˚C:0h	71	0.000	1.0000
4˚C:0h - 20˚C:0h	71	0.000	1.0000
10˚C:0h - 20˚C:0h	71	0.000	1.0000
4˚C:8h - 10˚C:8h	71	-0.753	0.9998
4˚C:8h - 20˚C:8h	71	-2.354	0.4501
10˚C:8h - 20˚C:8h	71	-1.600	0.9032
4˚C:24h - 10˚C:24h	71	-1.021	0.9967
4˚C:24h - 20˚C:24h	**71**	**-5.621**	**<.0001**
10˚C:24h - 20˚C:24h	**71**	**-4.600**	**0.0010**
4˚C:48h - 10˚C:48h	71	1.307	0.9755
4˚C:48h - 20˚C:48h	**71**	**-7.391**	**<.0001**
10˚C:48h - 20˚C:48h	**71**	**-8.698**	**<.0001**

Table 7. Multiple pairwise comparisons from the sugar kelp temperature storage trials with *V. parahaemolyticus.*

Multiple pairwise comparisons between all timepoints at each storage temperature using Tukey's HSD test. Significant comparisons are bolded.

Temp:Time – Temp:Time	df	t	p
4˚C:0h - 4˚C:8h	71	-0.290	1.0000
4˚C:0h - 4˚C:24h	71	0.443	1.0000
4˚C:0h - 4˚C:48h	71	2.108	0.6188
4˚C:8h - 4˚C:24h	71	0.656	0.9999
4˚C:8h - 4˚C:48h	71	2.145	0.5932
4˚C:24h - 4˚C:48h	71	1.489	0.9387
10˚C:0h - 10˚C:8h	71	-1.133	0.9921
10˚C:0h - 10˚C:24h	71	-0.698	0.9999
10˚C:0h - 10˚C:48h	**71**	**3.570**	**0.0293**
10˚C:8h - 10˚C:24h	71	0.389	1.0000
10˚C:8h - 10˚C:48h	**71**	**4.206**	**0.0040**
10˚C:24h - 10˚C:48h	**71**	**3.817**	**0.0140**
20˚C:0h - 20˚C:8h	71	-2.922	0.1557
20˚C:0h - 20˚C:24h	**71**	**-5.842**	**<.0001**
20˚C:0h - 20˚C:48h	**71**	**-6.155**	**<.0001**
20˚C:8h - 20˚C:24h	71	-2.611	0.2933
20˚C:8h - 20˚C:48h	71	-2.892	0.1663
20˚C:24h - 20˚C:48h	71	-0.281	1.0000

Table 8. ANOVA from the sugar kelp temperature storage trials with *E. coli.*
ANOVA results from a linear mixed effects model analyzing the effect of storage temperature (Temp), time in storage (Time), and the interaction between Temp and Time on pathogen load. Significant comparisons are bolded.

	Sum Sq	Mean Sq	Num df	Den df	F value	p value
Temp	**7.0631**	**3.5316**	**2**	**70.003**	**19.5504**	**1.796e-07*** **
Time	1.4282	0.4761	3	70.002	2.6354	0.05645
Temp:Time	**7.3891**	**1.2315**	**6**	**70.002**	**6.8175**	**1.008e-05*** **

Table 9. Multiple pairwise comparisons from the sugar kelp temperature storage trials with *E. coli.*
Multiple pairwise comparisons between all storage temperatures at each timepoint using Tukey's HSD test. Significant comparisons are bolded.

Temp:Time – Temp:Time	df	t	p
4˚C:0h - 10˚C:0h	70	0.000	1.0000
4˚C:0h - 20˚C:0h	70	0.000	1.0000
10˚C:0h - 20˚C:0h	70	0.000	1.0000
4˚C:8h - 10˚C:8h	70	-0.562	1.0000
4˚C:8h - 20˚C:8h	70	-1.040	0.9961
10˚C:8h - 20˚C:8h	70	-0.479	1.0000
4˚C:24h - 10˚C:24h	70	-0.442	1.0000
4˚C:24h - 20˚C:24h	70	-2.949	0.1466
10˚C:24h - 20˚C:24h	70	-2.507	0.3529
4˚C:48h - 10˚C:48h	70	-0.357	1.0000
4˚C:48h - 20˚C:48h	**70**	**-6.934**	**<.0001**
10˚C:48h - 20˚C:48h	**70**	**-6.250**	**<.0001**

Table 10. Multiple pairwise comparisons from the sugar kelp temperature storage trials with *E. coli.*

Multiple pairwise comparisons between all timepoints at each storage temperature using Tukey's HSD test. Significant comparisons are bolded.

Temp:Time – Temp:Time	df	t	p
4˚C:0h - 4˚C:8h	70	1.490	0.9384
4˚C:0h - 4˚C:24h	70	2.821	0.1943
4˚C:0h - 4˚C:48h	70	3.101	0.1027
4˚C:8h - 4˚C:24h	70	1.190	0.9881
4˚C:8h - 4˚C:48h	70	1.441	0.9508
4˚C:24h - 4˚C:48h	70	0.251	1.0000
10˚C:0h - 10˚C:8h	70	0.862	0.9993
10˚C:0h - 10˚C:24h	70	2.326	0.4685
10˚C:0h - 10˚C:48h	70	2.527	0.3412
10˚C:8h - 10˚C:24h	70	1.310	0.9752
10˚C:8h - 10˚C:48h	70	1.551	0.9201
10˚C:24h - 10˚C:48h	70	0.304	1.0000
20˚C:0h - 20˚C:8h	70	0.327	1.0000
20˚C:0h - 20˚C:24h	70	-0.477	1.0000
20˚C:0h - 20˚C:48h	**70**	**-4.651**	**0.0009**
20˚C:8h - 20˚C:24h	70	-0.719	0.9999
20˚C:8h - 20˚C:48h	**70**	**-4.453**	**0.0017**
20˚C:24h - 20˚C:48h	**70**	**-3.734**	**0.0182**

Table 11. ANOVA from the sugar kelp temperature storage trials with *L. monocytogenes.*

ANOVA results from a linear mixed effects model analyzing the effect of storage temperature (Temp), time in storage (Time), and the interaction between Temp and Time on pathogen load. Significant comparisons are bolded.

	Sum Sq	Mean Sq	Num df	Den df	F value	p value
Temp	**4.6958**	**2.3479**	**2**	**72**	**9.5687**	**0.0002065*** **
Time	**24.8529**	**8.2843**	**3**	**72**	**33.7624**	**9.794e-14*** **
Temp:Time	**17.0893**	**2.8482**	**6**	**72**	**11.6078**	**4.733e-09*** **

Table 12. Multiple pairwise comparisons from the sugar kelp temperature storage trials with *L. monocytogenes.*

Multiple pairwise comparisons between all storage temperatures at each timepoint using Tukey's HSD test. Significant comparisons are bolded.

Temp:Time – Temp:Time	df	t	p
4˚C:0h - 10˚C:0h	71	0.000	1.0000
4˚C:0h - 20˚C:0h	71	0.000	1.0000
10˚C:0h - 20˚C:0h	71	0.000	1.0000
4˚C:8h - 10˚C:8h	71	-0.858	0.9993
4˚C:8h - 20˚C:8h	71	-0.925	0.9986
10˚C:8h - 20˚C:8h	71	-0.066	1.0000
4˚C:24h - 10˚C:24h	71	-1.187	0.9884
4˚C:24h - 20˚C:24h	71	-0.997	0.9973
10˚C:24h - 20˚C:24h	71	0.190	1.0000
4˚C:48h - 10˚C:48h	71	-0.164	1.0000
4˚C:48h - 20˚C:48h	**71**	**7.745**	**<.0001**
10˚C:48h - 20˚C:48h	**71**	**7.910**	**<.0001**

Table 13. Multiple pairwise comparisons from the sugar kelp temperature storage trials with *L. monocytogenes.*

Multiple pairwise comparisons between all timepoints at each storage temperature using Tukey's HSD test. Significant comparisons are bolded.

Temp Time – Temp Time	df	t	p
4˚C:0h - 4˚C:8h	71	0.908	0.9988
4˚C:0h - 4˚C:24h	71	2.022	0.6774
4˚C:0h - 4˚C:48h	71	2.636	0.2804
4˚C:8h - 4˚C:24h	71	0.996	0.9973
4˚C:8h - 4˚C:48h	71	1.545	0.9221
4˚C:24h - 4˚C:48h	71	0.549	1.0000
10˚C:0h - 10˚C:8h	71	-0.052	1.0000
10˚C:0h - 10˚C:24h	71	0.694	0.9999
10˚C:0h - 10˚C:48h	71	2.452	0.3866
10˚C:8h - 10˚C:24h	71	0.667	0.9999
10˚C:8h - 10˚C:48h	71	2.239	0.5279
10˚C:24h - 10˚C:48h	71	1.572	0.9134
20˚C:0h - 20˚C:8h	71	-0.126	1.0000
20˚C:0h - 20˚C:24h	71	0.907	0.9988
20˚C:0h - 20˚C:48h	**71**	**11.295**	**<.0001**
20˚C:8h - 20˚C:24h	71	0.924	0.9986
20˚C:8h - 20˚C:48h	**71**	**10.215**	**<.0001**
20˚C:24h - 20˚C:48h	**71**	**9.291**	**<.0001**

Table 14. ANOVA from the sugar kelp temperature storage trials with *S. aureus.*
ANOVA results from a linear mixed effects model analyzing the effect of storage temperature (Temp), time in storage (Time), and the interaction between Temp and Time on pathogen load. Significant comparisons are bolded.

	Sum Sq	Mean Sq	Num df	Den df	F value	p value
Temp	1.5717	0.7859	2	102.99	2.9756	0.05542
Time	**20.0944**	**6.6981**	**3**	**103.14**	**25.3620**	**2.269e-12*** **
Temp:Time	1.8325	0.3054	6	102.99	1.1564	0.33556

Table 15. Multiple pairwise comparisons from the sugar kelp temperature storage trials with *S. aureus.*
Multiple pairwise comparisons between all sampling timepoints, regardless of storage temperature, using Tukey's HSD test. Significant comparisons are bolded.

Time	df	t	p
0h - 8h	**103**	**6.567**	**<.0001**
0h - 24h	**103**	**4.736**	**<.0001**
0h - 48h	**103**	**7.572**	**<.0001**
8h - 24h	103	-0.749	0.8769
8h - 48h	103	0.903	0.8035
24h - 48h	103	1.534	0.4209

Table 16. ANOVA from the sugar kelp temperature storage trials with *V. vulnificus.*
ANOVA results from a linear mixed effects model analyzing the effect of storage temperature (Temp), time in storage (Time), and the interaction between Temp and Time on pathogen load. Significant comparisons are bolded.

	Sum Sq	Mean Sq	Num df	Den df	F value	p value
Temp	3.0742	1.5371	2	72	3.0557	0.053241
Time	**12.0595**	**4.0198**	**3**	**72**	**7.9914**	**0.000114*** **
Temp:Time	6.6136	1.1023	6	72	2.1913	0.053543

Table 17. Multiple pairwise comparisons from the sugar kelp temperature storage trials with *V. vulnificus.*
Multiple pairwise comparisons between all sampling timepoints, regardless of storage temperature, using Tukey's HSD test. Significant comparisons are bolded.

Time	df	t	p
0h - 8h	71	-0.859	0.8261
0h - 24h	71	1.421	0.4904
0h - 48h	**71**	**4.112**	**0.0006**
8h - 24h	71	2.039	0.1835
8h - 48h	**71**	**4.446**	**0.0002**
24h - 48h	71	2.407	0.0850

Table 18. ANOVA from the rockweed temperature storage trials with *S. enterica.*
ANOVA results from a linear mixed effects model analyzing the effect of storage temperature (Temp), time in storage (Time), and the interaction between Temp and Time on pathogen load. Significant comparisons are bolded.

	Sum Sq	Mean Sq	Num df	Den df	F value	p value
Temp	**0.32199**	**0.16099**	**2**	**60.796**	**5.6774**	**0.005488****
Time	**1.22049**	**0.61024**	**2**	**62.351**	**21.5203**	**7.819e-08*****
Temp:Time	**0.48657**	**0.12164**	**4**	**60.796**	**4.2898**	**0.004017****

Table 19. Multiple pairwise comparisons from the rockweed temperature storage trials with *S. enterica.*
Multiple pairwise comparisons between all storage temperatures at each timepoint using Tukey's HSD test. Significant comparisons are bolded.

Temp:Time – Temp:Time	df	t	p
4˚C:0h - 10˚C:0h	61.0	0.000	1.0000
4˚C:0h - 20˚C:0h	61.0	0.000	1.0000
10˚C:0h - 20˚C:0h	61.0	0.000	1.0000
4˚C:24h - 10˚C:24h	61.0	-0.327	1.0000
4˚C:24h - 20˚C:24h	61.0	0.474	0.9999
10˚C:24h - 20˚C:24h	61.0	0.801	0.9964
4˚C:48h - 10˚C:48h	61.0	1.254	0.9404
4˚C:48h - 20˚C:48h	**61.0**	**5.255**	**0.0001**
10˚C:48h - 20˚C:48h	**61.0**	**4.001**	**0.0051**

Table 20. Multiple pairwise comparisons from the rockweed temperature storage trials with *S. enterica.*
Multiple pairwise comparisons between all timepoints at each storage temperature using Tukey's HSD test. Significant comparisons are bolded.

Temp:Time – Temp:Time	df	t	p
4˚C:0h - 4˚C:24h	61.9	2.748	0.1528
4˚C:0h - 4˚C:48h	61.0	1.362	0.9074
4˚C:24h - 4˚C:48h	61.9	-1.554	0.8252
10˚C:0h - 10˚C:24h	61.9	2.397	0.3041
10˚C:0h - 10˚C:48h	61.0	2.617	0.2013
10˚C:24h - 10˚C:48h	61.9	-0.103	1.0000
20˚C:0h - 20˚C:24h	**61.9**	**3.257**	**0.0446**
20˚C:0h - 20˚C:48h	**61.0**	**6.617**	**<.0001**
20˚C:24h - 20˚C:48h	61.9	2.543	0.2323

Table 21. ANOVA from the rockweed temperature storage trials with *V. parahaemolyticus.*
ANOVA results from a linear mixed effects model analyzing the effect of storage temperature (Temp), time in storage (Time), and the interaction between Temp and Time on pathogen load. Significant comparisons are bolded.

	Sum Sq	Mean Sq	Num df	Den df	F value	p value
Temp	0.66214	0.33107	2	60	2.6529	0.0787
Time	0.37898	0.18949	2	60	1.5184	0.2274
Temp:Time	0.47019	0.11755	4	60	0.9419	0.4461

Table 22. ANOVA from the rockweed temperature storage trials with *E. coli.*
ANOVA results from a linear mixed effects model analyzing the effect of storage temperature (Temp), time in storage (Time), and the interaction between Temp and Time on pathogen load. Significant comparisons are bolded.

	Sum Sq	Mean Sq	Num df	Den df	F value	p value
Temp	0.01386	0.00693	2	72	0.0512	0.9500899
Time	**2.29984**	**1.14992**	**2**	**72**	**8.5038**	**0.0004837*** **
Temp:Time	0.62148	0.15537	4	72	1.1490	0.3406102

Table 23. Multiple pairwise comparisons from the rockweed temperature storage trials with *E. coli.*
Multiple pairwise comparisons between all sampling timepoints, regardless of storage temperature, using Tukey's HSD test. Significant comparisons are bolded.

Time	df	t	p
0h - 24h	**70**	**3.514**	**0.0022**
0h - 48h	**70**	**3.626**	**0.0016**
24h - 48h	70	0.112	0.9931

Table 24. ANOVA from the rockweed temperature storage trials with *L. monocytogenes.*
ANOVA results from a linear mixed effects model analyzing the effect of storage temperature (Temp), time in storage (Time), and the interaction between Temp and Time on pathogen load. Significant comparisons are bolded.

	Sum Sq	Mean Sq	Num df	Den df	F value	p value
Temp	0.15290	0.07645	2	96	0.2145	0.80733
Time	**2.38400**	**1.19200**	**2**	**96**	**3.3444**	**0.03944***
Temp:Time	0.11871	0.02968	4	96	0.0833	0.98738

Table 25. Multiple pairwise comparisons from the rockweed temperature storage trials with *L. monocytogenes.*
Multiple pairwise comparisons between all sampling timepoints, regardless of storage temperature, using Tukey's HSD test. Significant comparisons are bolded.

Time	df	t	p
0h - 24h	96	2.100	0.0952
0h - 48h	96	2.358	0.0529
24h - 48h	96	0.258	0.9640

Table 26. ANOVA from the rockweed temperature storage trials with *S. aureus.*
ANOVA results from a linear mixed effects model analyzing the effect of storage temperature (Temp), time in storage (Time), and the interaction between Temp and Time on pathogen load. Significant comparisons are bolded.

	Sum Sq	Mean Sq	Num df	Den df	F value	p value
Temp	**1.2255**	**0.6128**	**2**	**70**	**8.1688**	**0.0006478*** **
Time	**21.5300**	**10.7650**	**2**	**70**	**143.5097**	**<2.2e-16*** **
Temp:Time	0.6896	0.1724	4	70	2.2983	0.0674197

Table 27. Multiple pairwise comparisons from the rockweed temperature storage trials with *S. aureus.*
Multiple pairwise comparisons between all storage temperatures, regardless of time in storage, using Tukey's HSD test. Significant comparisons are bolded.

Temp	df	t	p
4˚C - 10˚C	70	0.560	0.8419
4˚C - 20˚C	**70**	**3.747**	**0.0011**
10˚C - 20˚C	**70**	**3.187**	**0.0060**

Table 28. Multiple pairwise comparisons from the rockweed temperature storage trials with *S. aureus.*
Multiple pairwise comparisons between all sampling timepoints, regardless of storage temperature, using Tukey's HSD test. Significant comparisons are bolded.

Time	df	t	p
0h - 24h	**70**	**14.479**	**<.0001**
0h - 48h	**70**	**14.857**	**<.0001**
24h - 48h	70	0.378	0.9242

Table 29. ANOVA from the rockweed temperature storage trials with *V. vulnificus.*
ANOVA results from a linear mixed effects model analyzing the effect of storage temperature (Temp), time in storage (Time), and the interaction between Temp and Time on pathogen load. Significant comparisons are bolded.

	Sum Sq	Mean Sq	Num df	Den df	F value	p value
Temp	**2.0936**	**1.04680**	**2**	**70**	**9.2032**	**0.0002828*** **
Time	**3.7759**	**1.88797**	**2**	**70**	**16.5987**	**1.259e-06*** **
Temp:Time	**2.6021**	**0.65052**	**4**	**70**	**5.7193**	**0.0004840*** **

Table 30. Multiple pairwise comparisons from the rockweed temperature storage trials with *V. vulnificus.*

Multiple pairwise comparisons between all storage temperatures at each timepoint using Tukey's HSD test. Significant comparisons are bolded.

Temp:Time – Temp:Time	df	t	p
4˚C:0h - 10˚C:0h	70	0.000	1.0000
4˚C:0h - 20˚C:0h	70	0.000	1.0000
10˚C:0h - 20˚C:0h	70	0.000	1.0000
4˚C:24h - 10˚C:24h	70	0.747	0.9978
4˚C:24h - 20˚C:24h	70	-1.587	0.8088
10˚C:24h - 20˚C:24h	70	-2.334	0.3370
4˚C:48h - 10˚C:48h	**70**	**-4.023**	**0.0043**
4˚C:48h - 20˚C:48h	**70**	**-5.828**	**<.0001**
10˚C:48h - 20˚C:48h	70	-1.805	0.6790

Table 31. Multiple pairwise comparisons from the rockweed temperature storage trials with *V. vulnificus.*

Multiple pairwise comparisons between all timepoints at each storage temperature using Tukey's HSD test. Significant comparisons are bolded.

Temp:Time – Temp:Time	df	t	p
4˚C:0h - 4˚C:24h	70	1.996	0.5513
4˚C:0h - 4˚C:48h	**70**	**6.610**	**<.0001**
4˚C:24h - 4˚C:48h	**70**	**4.614**	**0.0006**
10˚C:0h - 10˚C:24h	70	2.743	0.1520
10˚C:0h - 10˚C:48h	70	2.586	0.2111
10˚C:24h - 10˚C:48h	70	-0.156	1.0000
20˚C:0h - 20˚C:24h	70	0.409	1.0000
20˚C:0h - 20˚C:48h	70	0.782	0.9970
20˚C:24h - 20˚C:48h	70	0.372	1.0000

Table 32. ANOVA from the sugar kelp drying method trials with *V. parahaemolyticus.*

ANOVA results from a linear mixed effects model analyzing the effect of drying method, product type, and the interaction between drying method and product type on pathogen load. Significant comparisons are bolded.

	Sum Sq	Mean Sq	Num df	Den df	F value	p value
Drying Method	0.187	0.187	1	29	1.494	0.2314
Product Type	**138.537**	**69.268**	**2**	**29**	**553.990**	**<2e-16*** **
Drying Method: Product Type	0.374	0.187	2	29	1.494	0.2412

Table 33. Multiple pairwise comparisons from the sugar kelp drying method trials with *V. parahaemolyticus.*
Multiple pairwise comparisons between all product types, regardless of drying method, using Tukey's HSD test. Significant comparisons are bolded.

Product Type	df	t	p
Post Dry - Dry Storage	29	1.497	0.3071
Post Dry – Wet	**29**	**-28.049**	**<.0001**
Dry Storage - Wet	**29**	**-29.546**	**<.0001**

Table 34. ANOVA from the sugar kelp drying method trials with *V. vulnificus.*
ANOVA results from a linear mixed effects model analyzing the effect of drying method, product type, and the interaction between drying method and product type on pathogen load. Significant comparisons are bolded.

	Sum Sq	Mean Sq	Num df	Den df	F value	p value
Drying Method	0.000	0.000	1	30	0.00	1
Product Type	**86.806**	**43.403**	**2**	**30**	**966.87**	**<2e-16*** **
Drying Method: Product Type	0.000	0.000	2	30	0.00	1

Table 35. Multiple pairwise comparisons from the sugar kelp drying method trials with *V. vulnificus.*
Multiple pairwise comparisons between all product types, regardless of drying method, using Tukey's HSD test. Significant comparisons are bolded.

Product Type	df	t	p
Post Dry - Dry Storage	29	0.000	1.0000
Post Dry – Wet	**29**	**-38.083**	**<.0001**
Dry Storage - Wet	**29**	**-38.083**	**<.0001**

Table 36. ANOVA from the sugar kelp drying method trials with *L. monocytogenes.*
ANOVA results from a linear mixed effects model analyzing the effect of drying method, product type, and the interaction between drying method and product type on pathogen load. Significant comparisons are bolded.

	Sum Sq	Mean Sq	Num df	Den df	F value	p value
Drying Method	0.0606	0.0606	1	30	0.2504	0.6204
Product Type	**25.0741**	**12.5370**	**2**	**30**	**51.8022**	**1.86e- 10*** **
Drying Method: Product Type	0.1212	0.0606	2	30	0.2504	0.7801

Table 37. Multiple pairwise comparisons from the sugar kelp drying method trials with *L. monocytogenes.*

Multiple pairwise comparisons between all product types, regardless of drying method, using Tukey's HSD test. Significant comparisons are bolded.

Product Type	df	t	p
Post Dry - Dry Storage	29	0.613	0.8143
Post Dry - Wet	**29**	**-8.493**	**<.0001**
Dry Storage - Wet	**29**	**-9.105**	**<.0001**

Table 38. ANOVA from the sugar kelp drying method trials with *E. coli.*

ANOVA results from a linear mixed effects model analyzing the effect of drying method, product type, and the interaction between drying method and product type on pathogen load. Significant comparisons are bolded.

	Sum Sq	Mean Sq	Num df	Den df	F value	p value
Drying Method	**2.014**	**2.014**	**1**	**29**	**17.707**	**0.0002268*** **
Product Type	**80.362**	**40.181**	**2**	**29**	**353.321**	**<2.2e-16*** **
Drying Method: Product Type	**4.027**	**2.014**	**2**	**29**	**17.707**	**9.431e-06*** **

Table 39. Multiple pairwise comparisons from the sugar kelp drying method trials with *E. coli.*

Multiple pairwise comparisons between all drying method and product type combinations of relevance using Tukey's HSD test. All air- and freeze-dried seaweed samples were compared to the same set of wet seaweed samples. Significant comparisons are bolded.

Drying Method:Product Type - Drying Method:Product Type	df	t	p
Air-Dry:Post Dry - Wet	**29**	**-17.357**	**<.0001**
Freeze-Dry:Post Dry - Wet	**29**	**-10.068**	**<.0001**
Air-Dry:Dry Storage - Wet	**29**	**-17.990**	**<.0001**
Freeze-Dry:Dry Storage - Wet	**29**	**-17.990**	**<.0001**
Air-Dry: Post Dry - Freeze-Dry:Post Dry	**29**	**-7.288**	**<.0001**
Air-Dry:Dry Storage - Freeze-Dry:Dry Storage	29	0.000	1.0000
Air-Dry:Post Dry- Air-Dry:Dry Storage	29	0.634	0.9874
Freeze-Dry:Post Dry - Freeze-Dry:Dry Storage	**29**	**7.922**	**<.0001**

Table 40. ANOVA from the sugar kelp drying method trials with *S. enterica.*
ANOVA results from a linear mixed effects model analyzing the effect of drying method, product type, and the interaction between drying method and product type on pathogen load. Significant comparisons are bolded.

	Sum Sq	Mean Sq	Num df	Den df	F value	p value
Drying Method	0.095	0.095	1	29	0.6427	0.4293
Product Type	**68.753**	**34.376**	**2**	**29**	**232.8538**	**<2e- 16*** **
Drying Method: Product Type	0.190	0.095	2	29	0.6427	0.5332

Table 41. Multiple pairwise comparisons from the sugar kelp drying method trials with *S. enterica.*
Multiple pairwise comparisons between all product types, regardless of drying method, using Tukey's HSD test. Significant comparisons are bolded.

Product Type	df	t	p
Post Dry - Dry Storage	29	0.982	0.5940
Post Dry - Wet	**29**	**-18.179**	**<.0001**
Dry Storage - Wet	**29**	**-19.161**	**<.0001**

Table 42. ANOVA from the sugar kelp drying method trials with *S. aureus.*
ANOVA results from a linear mixed effects model analyzing the effect of drying method, product type, and the interaction between drying method and product type on pathogen load. Significant comparisons are bolded.

	Sum Sq	Mean Sq	Num df	Den df	F value	p value
Drying Method	**1.315**	**1.315**	**1**	**27.000**	**13.806**	**0.0009344*** **
Product Type	**101.869**	**50.935**	**2**	**27.152**	**534.705**	**<2.2e-16*** **
Drying Method: Product Type	**2.723**	**1.362**	**2**	**27.000**	**14.293**	**5.839e-05*** **

Table 43. Multiple pairwise comparisons from the sugar kelp drying method trials with *S. aureus.*

Multiple pairwise comparisons between all drying method and product type combinations of relevance using Tukey's HSD test. All air- and freeze-dried seaweed samples were compared to the same set of wet seaweed samples. Significant comparisons are bolded.

Drying Method:Product Type - Drying Method:Product Type	df	t	p
Air-Dry:Post Dry - Wet	**27.1**	**-20.271**	**<.0001**
Freeze-Dry:Post Dry - Wet	**27.1**	**-13.967**	**<.0001**
Air-Dry:Dry Storage - Wet	**27.1**	**-22.251**	**<.0001**
Freeze-Dry:Dry Storage - Wet	**27.1**	**-22.251**	**<.0001**
Air-Dry: Post Dry - Freeze-Dry:Post Dry	**27.0**	**-6.647**	**<.0001**
Air-Dry:Dry Storage - Freeze-Dry:Dry Storage	27.0	0.000	1.0000
Air-Dry:Post Dry- Air-Dry:Dry Storage	27.0	2.087	0.3233
Freeze-Dry:Post Dry - Freeze-Dry:Dry Storage	**27.0**	**8.734**	**<.0001**

Table 44. ANOVA from the rockweed drying method trials with *V. parahaemolyticus.*

ANOVA results from a linear mixed effects model analyzing the effect of drying method, product type, and the interaction between drying method and product type on pathogen load. Significant comparisons are bolded.

	Sum Sq	Mean Sq	Num df	Den df	F value	p value
Drying Method	0.446	0.446	1	29	2.0793	0.1600
Product Type	**114.030**	**57.015**	**2**	**29**	**265.6487**	**<2e-16*** **
Drying Method: Product Type	0.893	0.446	2	29	2.0793	0.1433

Table 45. Multiple pairwise comparisons from the rockweed drying method trials with *V. parahaemolyticus.*

Multiple pairwise comparisons between all product types, regardless of drying method, using Tukey's HSD test. Significant comparisons are bolded.

Product Type	df	t	p
Post Dry - Dry Storage	29	2.272	0.0760
Post Dry – Wet	**29**	**-18.729**	**<.0001**
Dry Storage - Wet	**29**	**-21.001**	**<.0001**

Table 46. ANOVA from the rockweed drying method trials with *V. vulnificus.*
ANOVA results from a linear mixed effects model analyzing the effect of drying method, product type, and the interaction between drying method and product type on pathogen load. Significant comparisons are bolded.

	Sum Sq	Mean Sq	Num df	Den df	F value	p value
Drying Method	0.006	0.006	1	60.399	0.2195	0.6411
Product Type	**259.907**	**129.954**	**2**	**60.380**	**5096.8964**	**<2e-16*** **
Drying Method: Product Type	0.017	0.008	2	60.380	0.3244	0.7242

Table 47. Multiple pairwise comparisons from the rockweed drying method trials with *V. vulnificus.*
Multiple pairwise comparisons between all product types, regardless of drying method, using Tukey's HSD test. Significant comparisons are bolded.

Product Type	df	t	p
Post Dry - Dry Storage	60.5	0.564	0.8397
Post Dry – Wet	**60.0**	**-88.425**	**<.0001**
Dry Storage - Wet	**60.5**	**-84.818**	**<.0001**

Table 48. ANOVA from the rockweed drying method trials with *L. monocytogenes.*
ANOVA results from a linear mixed effects model analyzing the effect of drying method, product type, and the interaction between drying method and product type on pathogen load. Significant comparisons are bolded.

	Sum Sq	Mean Sq	Num df	Den df	F value	p value
Drying Method	0.109	0.109	1	29	0.9411	0.3400
Product Type	**67.218**	**33.609**	**2**	**29**	**290.6781**	**<2e-16*** **
Drying Method: Product Type	0.218	0.109	2	29	0.9411	0.4018

Table 49. Multiple pairwise comparisons from the rockweed drying method trials with *L. monocytogenes.*
Multiple pairwise comparisons between all product types, regardless of drying method, using Tukey's HSD test. Significant comparisons are bolded.

Product Type	df	t	p
Post Dry - Dry storage	29	1.400	0.3543
Post Dry - Wet	**29**	**-20.146**	**<.0001**
Dry Storage - Wet	**29**	**-21.546**	**<.0001**

Table 50. ANOVA from the rockweed drying method trials with *E. coli.*
ANOVA results from a linear mixed effects model analyzing the effect of drying method, product type, and the interaction between drying method and product type on pathogen load. Significant comparisons are bolded.

	Sum Sq	Mean Sq	Num df	Den df	F value	p value
Drying Method	**0.173**	**0.173**	**1**	**63**	**4.5949**	**0.03593***
Product Type	**205.028**	**102.514**	**2**	**63**	**2724.6673**	**<2e-16*****
Drying Method: Product Type	**0.346**	**0.173**	**2**	**63**	**4.5949**	**0.01372***

Table 51. Multiple pairwise comparisons from the rockweed drying method trials with *E. coli.*
Multiple pairwise comparisons between all drying method and product type combinations of relevance using Tukey's HSD test. All air- and freeze-dried seaweed samples were compared to the same set of wet seaweed samples. Significant comparisons are bolded.

Drying Method:Product Type - Drying Method:Product Type	df	t	p
Air-dry:Post Dry - Wet	**63**	**-45.481**	**<.0001**
Freeze-dry:Post Dry - Wet	**63**	**-41.768**	**<.0001**
Air-dry: Dry Storage - Wet	**63**	**-46.635**	**<.0001**
Freeze-dry:Dry Storage - Wet	**63**	**-46.635**	**<.0001**
Air-dry:Post dry - Freeze-dry:Post Dry	**63**	**-3.713**	**0.0056**
Air-dry:Dry Storage - Freeze-dry:Dry Storage	63	0.000	1.0000
Air-dry:Post Dry - Air-dry:Dry Storage	63	1.154	0.8564
Freeze-dry:Post Dry - Freeze-dry:Dry Storage	**63**	**4.867**	**0.0001**

Table 52. ANOVA from the rockweed drying method trials with *S. enterica.*
ANOVA results from a linear mixed effects model analyzing the effect of drying method, product type, and the interaction between drying method and product type on pathogen load. Significant comparisons are bolded.

	Sum Sq	Mean Sq	Num df	Den df	F value	p value
Drying Method	0.269	0.269	1	66	1.8029	0.18397
Product Type	**189.762**	**94.881**	**2**	**66**	**634.8688**	**<2e-16*****
Drying Method: Product Type	**1.193**	**0.597**	**2**	**66**	**3.9920**	**0.02309***

Table 53. Multiple pairwise comparisons from the rockweed drying method trials with *S. enterica.*

Multiple pairwise comparisons between all drying method and product type combinations of relevance using Tukey's HSD test. All air- and freeze-dried seaweed samples were compared to the same set of wet seaweed samples. Significant comparisons are bolded.

Drying Method:Product Type - Drying Method:Product Type	df	t	p
Air-dry:Post Dry - Wet	**63**	**-21.801**	**<.0001**
Freeze-dry:Post Dry - Wet	**63**	**-18.757**	**<.0001**
Air-dry: Dry Storage - Wet	**63**	**-22.731**	**<.0001**
Freeze-dry:Dry Storage - Wet	**63**	**-23.450**	**<.0001**
Air-dry:Post dry - Freeze-dry:Post Dry	**63**	**-3.045**	**0.0381**
Air-dry:Dry Storage - Freeze-dry:Dry Storage	63	0.719	0.9789
Air-dry:Post Dry - Air-dry:Dry Storage	63	0.930	0.9373
Freeze-dry:Post Dry - Freeze-dry:Dry Storage	**63**	**4.693**	**0.0002**

Table 54. ANOVA from the rockweed drying method trials with *S. aureus.*

ANOVA results from a linear mixed effects model analyzing the effect of drying method, product type, and the interaction between drying method and product type on pathogen load. Significant comparisons are bolded.

	Sum Sq	Mean Sq	Num df	Den df	F value	p value
Drying Method	**3.965**	**3.965**	**1**	**28.056**	**13.383**	**0.00104****
Product Type	**101.443**	**50.722**	**2**	**28.054**	**171.195**	**<2.2e-16*****
Drying Method: Product Type	**7.773**	**3.886**	**2**	**28.054**	**13.118**	**9.51e-05*****

Table 55. Multiple pairwise comparisons from the rockweed drying method trials with *S. aureus.*

Multiple pairwise comparisons between all drying method and product type combinations of relevance using Tukey's HSD test. All air- and freeze-dried seaweed samples were compared to the same set of wet seaweed samples. Significant comparisons are bolded.

Drying Method:Product Type - Drying Method:Product Type	df	t	p
Air-dry:Post Dry - Wet	**28.0**	**-12.112**	**<.0001**
Freeze-dry:Post Dry - Wet	**28.0**	**-5.751**	**<.0001**
Air-dry: Dry Storage - Wet	**28.0**	**-13.059**	**<.0001**
Freeze-dry:Dry Storage - Wet	**28.1**	**-12.301**	**<.0001**
Air-dry:Post dry - Freeze-dry:Post Dry	**28.0**	**-6.361**	**<.0001**
Air-dry:Dry Storage - Freeze-dry:Dry Storage	28.1	-0.076	1.0000
Air-dry:Post Dry - Air-dry:Dry Storage	28.0	0.947	0.9304
Freeze-dry:Post Dry - Freeze-dry:Dry Storage	**28.1**	**6.850**	**<.0001**

Data on Natural Levels of Pathogen Contamination

Table 56. Detection frequency and average bacterial load of natural pathogen contamination on seaweed in various stages of processing.

Data presented represent pathogenic colonies recovered from control samples of seaweed across all of my experimental trials. Recovery events were organized by seaweed and pathogen species, only. Data was not organized based on the temperature storage or drying treatment the control samples may have undergone. The percent detection represents the frequency of controls that contained pathogen out of the total number of controls sampled (n). The average cfu/g represents the average pathogen load across control samples from which pathogen was recovered, not across all control sampled.

	L. monocytogenes		*S. enterica*		*S. aureus*	
	% detection	Avg. cfu/g ± std. dev.	% detection	Avg. cfu/g ± std. dev.	% detection	Avg. cfu/g ± std. dev.
Sugar Kelp	50 n=34	268 ± 222	6 n=34	175 ± 177	32 n=41	331 ± 316
Rockweed	0 n=38	0	9 n=33	67 ± 29	77 n=31	13177 ± 61608
	V. parahaemolyticus		*V. vulnificus*		*E. coli*	
	% detection	Avg. cfu/g ± std. dev.	% detection	Avg. cfu/g ± std. dev.	% detection	Avg. cfu/g ± std. dev.
Sugar Kelp	0 n=34	0	12 n=34	2100 ± 3903	38 n=34	200 ± 210
Rockweed	4 n=28	3600	17 n=36	200 ± 228	22 n=36	850 ± 838

Permits and Approvals

Ram Island Farm Lease:
https://www.maine.gov/dmr/aquaculture/leases/decisions/documents/UniversityofNewEnglandSACORIxnortheastofRamIslandSacoBaySaco_DecisionHistory.pdf

Wood Island Farm Lease:
file:///Users/jessicavorse/Downloads/CBYR121_SignedLicense.pdf

Approval for BSL-2 outdoor greenhouse for air-drying:

UNE UNIVERSITY OF NEW ENGLAND

Institutional Biosafety Committee
James Vaughn, Chair
George Allen, Vice Chair

Biddeford Campus
11 Hills Beach Road
Biddeford, ME 04005
(207)602-2244 T
(207)602-5905 F

Portland Campus
716 Stevens Avenue
Portland, ME 04103

TO:	Kristin Burkholder, Ph.D
FROM:	James Vaughn, Ph.D. Chair, Institutional Biosafety Committee
DATE:	January 21, 2020
RE:	IBC Approval
Protocol # & Title:	112519-011; Effect of processing conditions on microbiological safety of edible seaweed

Notice of IBC Review - APPROVAL

The project identified above has been reviewed by the University of New England's Institutional Biosafety Committee (IBC). This approval is based on the assumption that the materials, including changes/clarifications that you submitted to the IBC, contain a complete and accurate description of all the ways in which you will conduct your research.

A. Approved BSL/ABSL:
☒ BSL/ABSL-1 ☒ BSL/ABSL-2

B. PI must comply with *NIH Guidelines for Research Involving Recombinant DNA Molecules* (NIH Guidelines):
☒ Yes ☐ Not Applicable to this protocol

C. Select Agents Approved for Use (applies only to those listed in the protocol):
☐ Yes ☐ No ☒ Not Applicable to this protocol

D. Controlled Substances Approved for Use (applies only to those listed in the protocol):
☐ Yes ☐ No ☒ Not Applicable to this protocol

E. This approval is given with the following specific conditions:

1. No additional conditions.

F. This approval is given with the following general conditions:

1. You are approved to conduct this research only during the period of approval cited below;
2. You will conduct the research according to the plans and protocol submitted;
3. PI(s) should comply with the guidelines established in the *Biosafety in Microbiological and Biomedical Laboratories (BMBL) 5th Edition*;

   Note: PI(s) **must** comply with the *BMBL, 5th* if they are receiving Federal funding.
4. You will immediately inform the Director of Research Integrity of any injuries or adverse research events involving research subjects, faculty/staff or students, as well as any unexpected research animal illnesses or deaths (if applicable);
5. You will immediately request approval from the IBC for any proposed changes in your research, and you will not initiate any changes until they have been reviewed and approved by the IBC;
6. All personnel that are required to comply with UNE's Occupational Health and Safety Program, NIH/CDC's (or other Federal Agency's) Occupational Health Plan or other related health/medical requirements have done so **before** the onset of research activities. PI is responsible for maintaining appropriate documentation of this;
7. **You must submit an Annual Review Form to the IBC at least 60 days prior to the dates listed below.**
8. **If your research is anticipated to continue beyond the 3-year IBC approval date, you must submit a complete 3-Year Renewal to the IBC at least 60 days prior to the expiration date.**

G. General Safety Requirements:

1. Accidents, injuries or illness resulting from the use of toxic, biological, or radioactive substances must be reported to the Director of Research Integrity and the Office of Environmental Safety and Health immediately.
2. Any injuries or near injuries to researchers, staff or students that occur in the course of your protocol must also be immediately reported to the Director of Research Integrity.
3. Appropriate protective equipment and procedures for use and handling of toxic, biological, or radioactive substances must be maintained at all times.
4. Appropriate BSL's and/or ABSL's will be maintained at all times, including the use of appropriate biosafety cabinets.

The University appreciates your efforts to conduct research in compliance with the federal regulations and University Policies for the safe use of biological materials and agents.

3-Year Approval Period: **January 21, 2020 – January 20, 2023**

Annual Review Dates: **Januray 20, 2021 & January 20, 2022**

Sincerely,

James Vaughn, Ph.D.
Chair, Institutional Biosafety Committee