Journal of Applied Phycology (2023) 35:1331–1346
https://doi.org/10.1007/s10811-023-02937-w

Effect of post-harvest processing methods on the microbial safety of edible seaweed

Jessica G. Vorse[1] · Colleen T. Moody[2] · Lyle C. Massoia[2] · Jennifer J. Perry[3] · Kristin M. Burkholder[2] · Carrie J. Byron[1]

Received: 26 October 2022 / Revised and accepted: 25 February 2023 / Published online: 30 March 2023

Abstract
The American seaweed industry is growing, primarily into the edible sector, and 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 research included: *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 can lead to replication while storage at 4˚C and 10˚C halted the replication of focal pathogens. Both air-drying and freeze-drying produced significant log scale reductions in surface pathogen load for all focal pathogens. Additionally, air-drying reduced pathogen load more than freeze-drying for a majority of pathogens and 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.

Keywords Edible Macroalgae · Food Safety · Bacterial Pathogens · Aquaculture · Processing

Introduction

Edible seaweed industry

The global seaweed market has been estimated to increase 8–10% annually, with edible seaweed products making up an estimated 85% of global seaweed production. Asian countries dominate the market; however, the United States ranks in the top 10 countries for both importing and exporting (Piconi et al. 2020). Considering the United States nascent involvement in a globally expanding industry, there is notable potential for the growth of a lucrative domestic edible seaweed market. 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, Maine's farmed seaweed has increased by over 30-fold with a total harvest of 225,502 wet kg of sugar kelp, valued at US$301,285.60 for the 2020 season (Piconi et al. 2020; ME DMR 2021). 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 US$13.4 million to the state economy (Piconi et al. 2020). Not included in these estimates is another important species

✉ Carrie J. Byron
cbyron@une.edu

[1] School of Marine and Environmental Programs, University of New England, 11 Hills Beach Road, Biddeford, ME 04005, USA

[2] School of Biological Sciences, University of New England, 11 Hills Beach Road, Biddeford, ME 04005, USA

[3] School of Food and Agriculture, University of Maine, 5763 Rogers Hall, Orono, ME 04469, USA

in Maine's seaweed economy—rockweed (*Ascophyllum nodosum*). Although rockweed is not always considered an edible seaweed, it is consumed directly in teas, spices, and as an ingredient in other specialty food items, and therefore can be considered edible. It has an additional important role in the food industry as a fertilizer ingredient. 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 US$590,927 (ME DMR 2022).

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 processing 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 (U.S. FDA 2011, 2018). The HARPC is a regulation that applies across food industries; however, there are more specific FDA regulations that individual industries, like fresh 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 28 August 2020). 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" (U.S. FDA 2021). 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 (U.S. FDA 2020, 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 those 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, state-level regulatory frameworks for seaweed, if present at all, may not be the most appropriate. For example, the Connecticut Sea Grant (CSG), in partnership with the Connecticut Department of Agriculture Bureau of Aquaculture (DABA), developed 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 provide guidance and specify regulation for industry (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 Seafood HACCP, they are reminiscent of shellfish regulation. While compliance with 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) reported 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 when compared to shellfish, which are known to concentrate pathogens from the surrounding waters (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 *V. vulnificus* or *V. 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 *S. 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 or drying. One study found that when a homogenous mixture of five seaweed species were oven dried at 50˚C, *E. coli* and *E. coli* O157:H7 died off but were detected in the dried product 72 h later (Swinscoe et al. 2020). When drying occurred between 50–60˚C, *V. parahaemolyticus* was undetectable but *L. monocytogenes* remained detectable (Swinscoe et al. 2020). Alternatively, seaweed subject to secondary processing is most often air-dried for dry storage or blanched for frozen 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. 2019; 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. For example, no enterococci, coliforms, pathogenic vibrios, or *Listeria monocytogenes* were found on a small sample of fresh and frozen *S. latissima* and *A. esculenta* grown in Norway (Blikra et al. 2019). Similarly, there was no recovery of *Vibrio* spp. *L. monocytogenes*, *Salmonella* spp., or *S. aureus* from raw, blanched, and fermented *S. latissima* harvested off the coast of Maine (Akomea-Frempong et al. 2021). One presumptive *Vibrio* sp. was originally detected on a raw sample but was no-longer detectable after fermentation. Lastly, no human pathogenic strains of *Salmonella*, *E. coli*, *S. aureus* or *L. monocytogenes* were found on *S. latissima* sampled fresh/frozen, dried, and re-hydrated from Scotland, except for one sample of *A. esculenta* which harbored *L. monocytogenes*, likely introduced after harvest (Lytou et al. 2021).

We can conclude from the aforementioned research that foodborne 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 doses ≤ 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 and Frank 2007). Furthermore, if edible seaweed is not properly handled post-harvest, even low levels of contaminating pathogens could replicate to achieve an infective dose. 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 and 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), representing refrigeration, failed refrigeration, and cool ambient temperatures, respectively. In addition, we tested two different drying methods (air- and freeze-drying) of relevance to the seaweed industry. 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 foodborne 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 foodborne pathogens in the United States with some having specific associations with seafood products (Bintsis 2017). These experiments were designed in close collaboration with seaweed industry stakeholders, so our experimental designs mimicked real world processing conditions as closely as

possible. 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 & methods

Bacterial strains

Multi-strain cocktails of *Listeria monocytogenes, Salmonella enterica, Vibrio vulnificus, Vibrio parahaemolyticus, Staphylococcus aureus* and *Escherichia coli* were used for kelp inoculations (Table 1). Two to four strains of each species were combined into individual species cocktails moments before experimental inoculation. Stock cultures of each individual strain were maintained at -80˚C. Prior to preparing the inoculum cocktail, cultures of each strain 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). These liquid overnight cultures were used to make our inoculum cocktails by direct addition to the inoculum solution immediately preceding seaweed inoculation.

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 (Online Resource 1).

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

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

To recover focal pathogens from seaweed after our experimental trials, plating was performed using selective and differential media, as follows: *L. monocytogenes* plated on BD Palcam, *S. enterica* plated on HIMEDIA XLD, *V. vulnificus* and *V. parahaemolyticus* plated on CHROMagar *Vibrio, S. aureus* plated on Criterion Baird Parker Agar and *E. coli* plated on CHROMagar O157 (Table 1). The manufacturer's protocols were followed for each media type regarding media preparation and color-based colony identification.

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 (43.469593, -70.349921) and CBYR121 (43.45511, -70.33617) and were harvested from long-lines at the hold fast with a knife. Rockweed samples were wild harvested with a knife 40.64 cm 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 min 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 challenge tests

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 equivalent concentrations of 2–4 strains of an individual bacterial species to achieve a total cocktail concentration of 1×10^6 cfu g^{-1}. 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 1 h incubation all samples were drained and 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 as soon as the inoculation period was over, 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 Dulbecco's phosphate-buffered saline and plated via bead surface spreading 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 (Wesche and Ryser 2013). The results are reported in colony forming units per gram of kelp (cfu g^{-1}). Temperature challenge tests 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 challenge tests were completed in Spring 2021 and rockweed temperature challenge tests 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 all samples were drained and 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 moved to their drying treatments as soon as the inoculation period was over to be 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 screens. 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 (Online Resource 2). Freeze-dry samples were cut with scissors from 25 to 5 g ± 0.5, added to individual glass vials and placed in a Labconco freeze dryer for 48 h. 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 (post-dry) while the other half were vacuum sealed using a Greyon model #E1600-C and stored on the lab bench at room temperature (dry-stored) undergoing bacterial isolation on their 40^{th} 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 ~ 9 am – 3 pm 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 using an Aqualab Pre meter calibrated with 0.76 standard. Readings for each sample were taken in duplicate and averaged.

Statistical analysis

The limit of detection (LOD) for our plating method was 100 bacterial cells, or log 2 cfu g^{-1} 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^{-1} prior to conducting the analysis. All analyses were performed in R v. 4.0.2 (R Core Team 2020).

Temperature challenge tests

A linear mixed effects model was used to assess pathogen load as a function of time in storage (0 h, 8 h, 24 h, 48 h) and storage temperature (4˚C, 10˚C, 20˚C), where trial replicate served as a random variable. When significance was found, Tukey's HSD test was used to perform multiple pairwise comparisons.

Drying method trials

A linear mixed effects model was used to assess pathogen load as a function of drying method (air, freeze) and product type (wet, post-dry, dry-storage), with trial replicate again serving as a random variable. When significance was found, multiple pairwise comparisons were performed using Tukey's HSD test.

Results

Temperature challenge tests

The goal of the temperature challenge tests 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 h.

Sugar kelp

S. enterica Temperature ($F_{2,70.020} = 19.063$, $p < 0.001$) and its interaction with time ($F_{6,70.018} = 3.828$, $p < 0.01$) impacted the *S. enterica* population on sugar kelp, with no effect from time ($F_{3,70.019} = 1.292$, $p = 0.284$) (Fig. 1A). 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 both 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

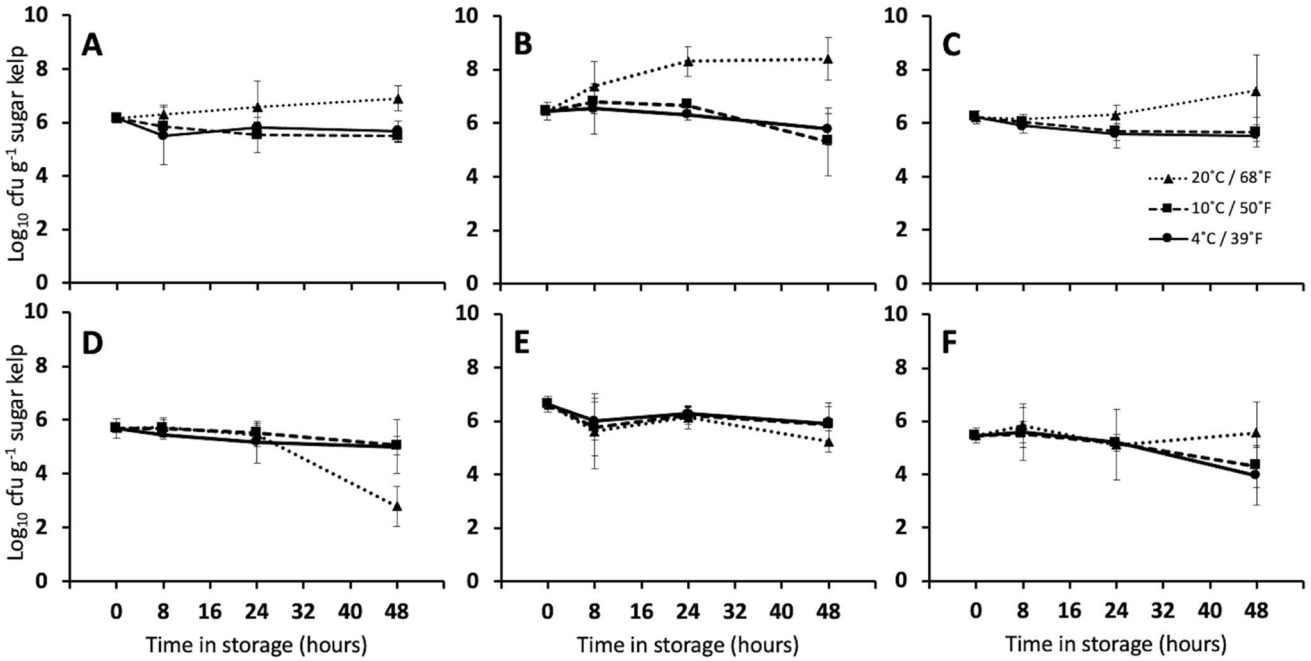

Fig. 1 Effect of temperature storage on pathogen load of sugar kelp. Pathogen load was evaluated as the $\log_{10}$ colony forming units per gram of kelp, shown on the y-axis. Time kelp spent in storage (hours) is shown on the x-axis. The line patterns represent our three storage temperatures: 4˚C (solid), 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

timepoints. Additionally, there were no significant changes in *S. enterica* load over time at any storage temperature tested.

V. parahaemolyticus Temperature ($F_{2,71}=42.436$, $p<0.001$), time ($F_{3,71}=5.463$, $p<0.01$) and their interaction ($F_{6,71}=10.695$, $p<0.001$) impacted the *V. parahaemolyticus* population on sugar kelp (Fig. 1B). 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 ($F_{2,70.003}=19.550$, $p<0.001$) and its interaction with time ($F_{6,70.002}=6.818$, $p<0.001$) impacted the *E. coli* population on sugar kelp, with no effect from time ($F_{3,70.002}=2.635$, $p=0.056$) (Fig. 1C). 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 ($F_{2,72}=9.569$, $p<0.001$), time ($F_{3,72}=33.762$, $p<0.001$), and their interaction ($F_{6,72}=11.608$, $p<0.001$) impacted the *L. monocytogenes* population on sugar kelp (Fig. 1D). 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 *Listeria* load on kelp stored at 20˚C for 48 h 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 ($F_{3,103.14}=25.362$, $p<0.001$) impacted the *S. aureus* population on sugar kelp, with no effect from temperature ($F_{2,102.99}=2.976$, $p=0.055$) or interaction ($F_{6,102.99}=1.156$, $p=0.336$) (Fig. 1E). At all temperatures tested, *S. aureus* levels at 0 h were significantly higher than

on kelp sampled at 8 h ($p < 0.001$), 24 h ($p < 0.001$), and 48 h ($p < 0.001$).

V. vulnificus Time ($F_{3,72} = 7.991$, $p < 0.001$) impacted the *V. vulnificus* population on sugar kelp, with no effect from temperature ($F_{2,72} = 3.056$, $p = 0.053$) or interaction ($F_{6,72} = 2.191$, $p = 0.054$) (Fig. 1F). 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.

Rockweed

S. enterica Temperature ($F_{2,60.796} = 5.677$, $p < 0.01$) and time ($F_{2,62.351} = 21.520$, $p < 0.001$), and their interaction ($F_{4,60.796} = 4.290$, $p < 0.01$) impacted the *S. enterica* population on rockweed (Fig. 2A). *S. enterica* load was significantly lower at both 24 h ($p < 0.05$) and 48 h ($p < 0.001$) when compared to 0 h. Additionally, rockweed stored at 20˚C for 48 h had a significantly lower *S. enterica* load than rockweed stored at both 4˚C ($p < 0.001$) and 10˚C ($p < 0.01$) for 48 h. Conversely, the pathogen load on rockweed 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 temperature ($F_{2,60} = 2.653$, $p = 0.079$), time ($F_{2,60} = 1.518$, $p = 0.227$) or their interaction ($F_{4,60} = 0.942$, $p = 0.446$) on the *V. parahaemolyticus* load on rockweed (Fig. 2B). Pathogen load remained constant across time and between storage temperatures.

E. coli Time ($F_{2,72} = 8.504$, $p < 0.001$) impacted *E. coli* population on rockweed, with no effect from temperature ($F_{2,72} = 0.051$, $p = 0.950$) or interaction ($F_{4,72} = 1.149$, $p = 0.341$) (Fig. 2C). This significance was explained by an overall decrease in pathogen load across time, regardless of storage temperature. The *E. coli* load on rockweed at both 24 h ($p < 0.01$) and 48 h ($p < 0.01$) was significantly lower than at 0 h.

L. monocytogenes The population of *L. monocytogenes* on rockweed decreased with time ($F_{2,96} = 3.344$, $p < 0.05$), with no effect from temperature ($F_{2,96} = 0.215$, $p = 0.807$) or interaction ($F_{4,96} = 0.083$, $p = 0.987$); however, multiple pairwise comparisons revealed no significant interactions between any of the sampling timepoints (Fig. 2D). Additionally, there were no significant differences in pathogen load between any of the storage temperatures.

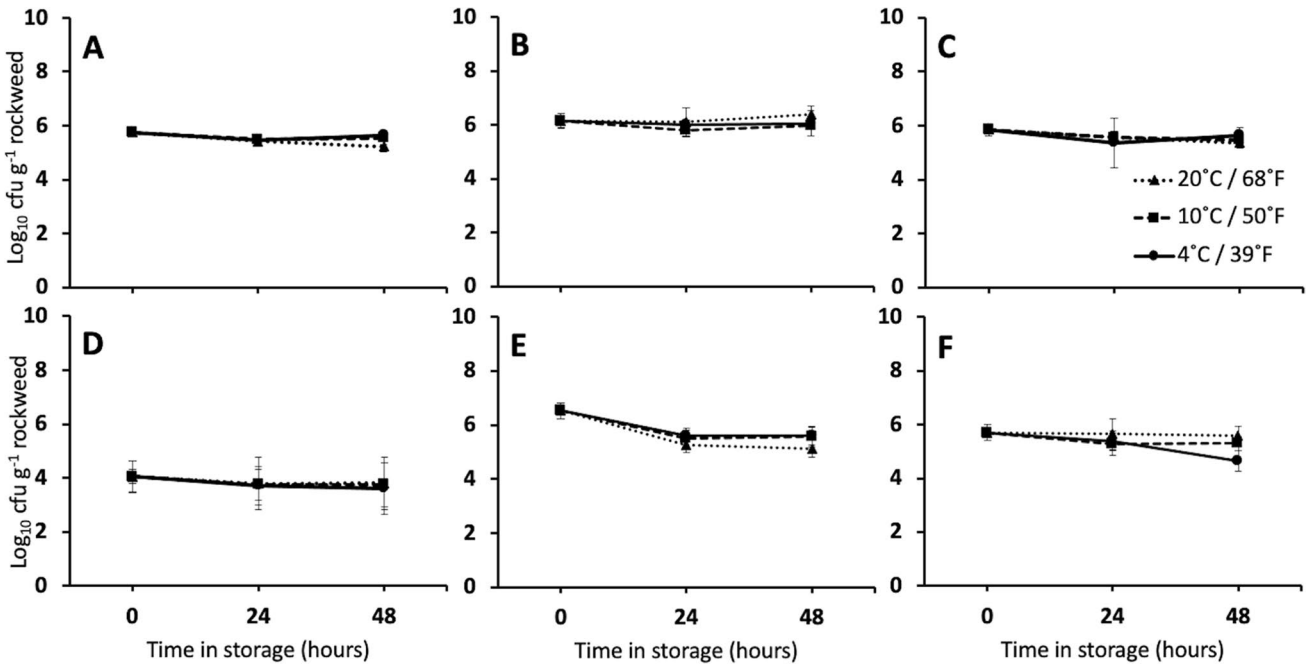

Fig. 2 Effect of temperature storage on pathogen load of rockweed. Pathogen load was evaluated as the $\log_{10}$ colony forming units per gram of kelp, shown on the y-axis. Time kelp spent in storage (hours) is shown on the x-axis. The line patterns represent our three storage temperatures: 4˚C (solid), 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

S. aureus Temperature ($F_{2,70}=8.169$, $p<0.001$) and time ($F_{2,70}=143.510$, $p<0.001$) impacted the *S. aureus* population on rockweed, with no interaction ($F_{4,70}=2.298$, $p=0.067$) (Fig. 2E). While we observed a numerical decrease in *S. aureus* at all storage temperatures, the decrease at 20 ˚C reached statistical significance. The *S. aureus* load on rockweed 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 rockweed stored at 20˚C was significantly lower than on rockweed stored at both 4˚C ($p<0.01$) and 10˚C ($p<0.01$), regardless of sampling time.

V. vulnificus Temperature ($F_{2,70}=9.203$, $p<0.001$), time ($F_{2,70}=16.599$, $p<0.001$) and their interaction ($F_{4,70}=5.719$, $p<0.001$) impacted *V. vulnificus* population on rockweed (Fig. 2F). *V. vulnificus* load significantly declined across time on rockweed 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, rockweed stored at 4˚C for 48 h contained significantly lower *V. vulnificus* numbers than rockweed 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 *V. vulnificus* load across time or between samples.

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 and rockweed. Pathogen load was evaluated on wet, post-dry and dry-stored samples (referred to as product type). All seaweed was dried to a water activity (a_w) of 0.49—0.60 (Fig. 3).

Sugar kelp

V. parahaemolyticus Product type ($F_{2,29}=553.990$, $p<0.001$) impacted *V. parahaemolyticus* population on sugar kelp, with no effect from drying method ($F_{1,29}=1.494$, $p=0.231$) or interaction ($F_{2,29}=1.494$, $p=0.241$) (Fig. 4A). 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. Although there was not a significant difference between the pathogen load of post-dry kelp and dry-stored kelp due to our LOD, we observed a numerical reduction in pathogen load due to dry storage, as *V. parahaemolyticus* was un-recoverable on dry-stored samples for both drying methods.

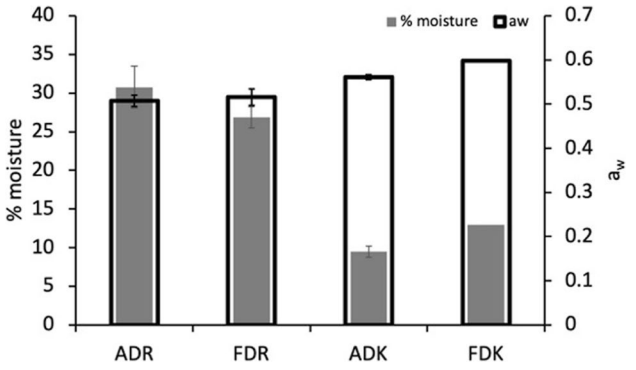

Fig. 3 Moisture content and a_w readings of air- and freeze-dried rockweed and sugar kelp. The left y-axis represents % moisture content and the right y-axis represents water activity ($a_w \pm 0.01$). The x-axis represents the mean value from all samples in a treatment group: air-dried rockweed (ADR, $n=8$), 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

V. vulnificus Product type ($F_{2,30}=966.87$, $p<0.001$) impacted the *V. vulnificus* population on sugar kelp, with no effect from drying method ($F_{1,30}=0.00$, $p=1$) or interaction ($F_{2,30}=0.00$, $p=1$) (Fig. 4B). 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 ($F_{2,30}=51.802$, $p<0.001$) impacted the *L. monocytogenes* population on sugar kelp, with no effect from drying method ($F_{1,30}=0.250$, $p=0.620$) or interaction ($F_{2,30}=0.250$, $p=0.780$) (Fig. 4C). 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.

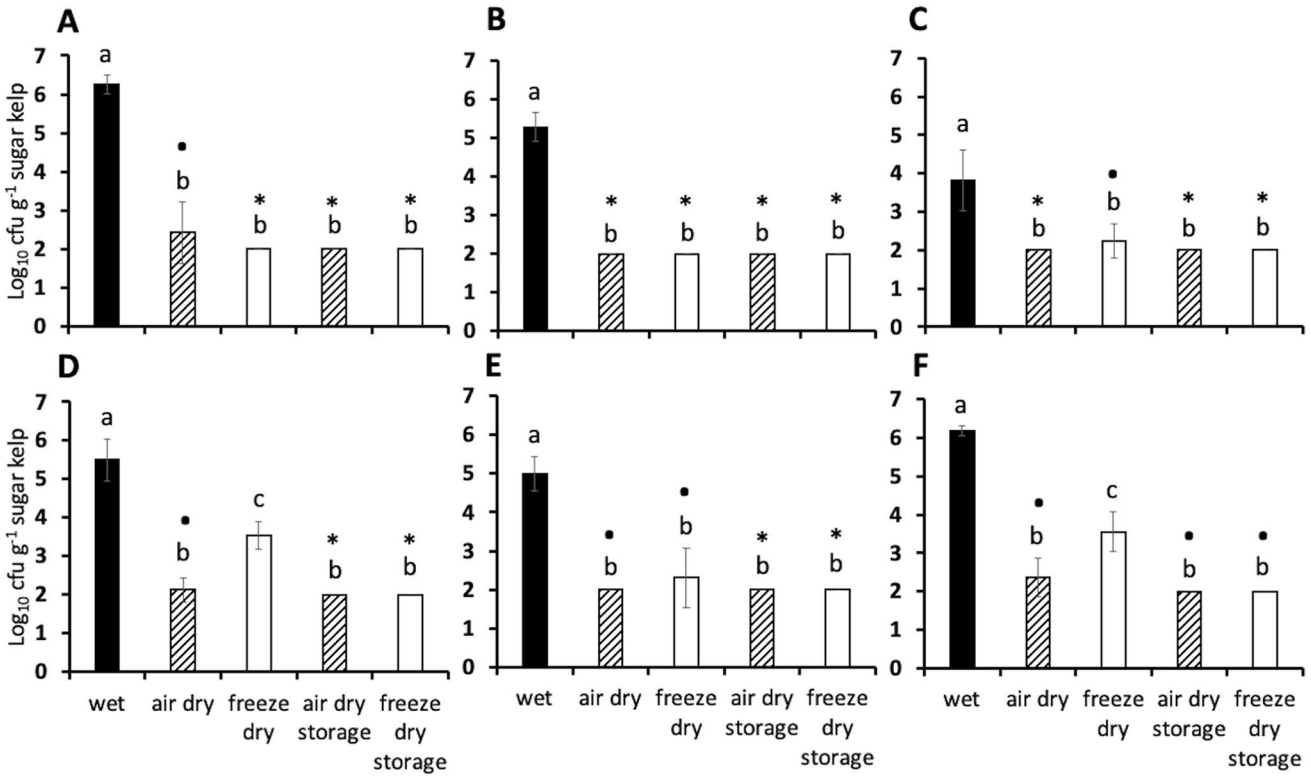

Fig. 4 Effect of drying method on pathogen load of sugar kelp. Pathogen load was evaluated as the $\log_{10}$ colony forming units per gram of kelp, shown on the y-axis. The product type sampled is shown on the x-axis: "wet" represents kelp sampled before any drying occurred, "air/freeze-dry" represents 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 letters identify significant differences ($p<0.001$) between treatments. The LOD of our sampling method was log 2 (100 cfu g^{-1}). 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

E. coli Drying method ($F_{1,29}=17.707$, $p<0.001$), product type ($F_{2,29}=353.321$, $p<0.001$), and their interaction ($F_{2,29}=17.707$, $p<0.001$) impacted the *E. coli* population on sugar kelp (Fig. 4D). 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 ($F_{2,29}=232.854$, $p<0.001$) impacted the *S. enterica* population on sugar kelp, with no effect from drying method ($F_{1,29}=0.643$, $p=0.429$) or interaction ($F_{2,29}=0.643$, $p=0.533$) (Fig. 4E). 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, 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 ($F_{1,27}=13.806$, $p<0.001$), product type ($F_{2,27.152}=534.705$, $p<0.001$), and their interaction ($F_{2,27}=14.293$, $p<0.001$) impacted the *S. aureus* population on sugar kelp (Fig. 4F). 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 ($p < 0.001$).

Rockweed

V. parahaemolyticus Product type ($F_{2,29} = 265.649$, $p < 0.001$) impacted the *V. parahaemolyticus* population on rockweed, with no effect from drying method ($F_{1,29} = 2.079$, $p = 0.160$) or interaction ($F_{2,29} = 2.079$, $p = 0.143$) (Fig. 5A). This significance was explained by an overall reduction in pathogen load as a result of drying. The *V. parahaemolyticus* load on wet rockweed was > 3 log higher than on both post-dry ($p < 0.001$) and dry-stored rockweed ($p < 0.001$), regardless of drying method. Though there was not a significant difference between the pathogen load of post-dry rockweed and dry-stored rockweed due to our LOD, 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 ($F_{2,60.380} = 5096.896$, $p < 0.001$) impacted the *V. vulnificus* population on rockweed, with no effect from drying method ($F_{1,60.399} = 0.220$, $p = 0.641$) or interaction ($F_{2,60.380} = 0.324$, $p = 0.724$) (Fig. 5B). This significance was explained by a reduction in pathogen load as a result of drying. The *V. vulnificus* load on wet rockweed was > 4 log higher than on both post-dry ($p < 0.001$) and dry-stored rockweed ($p < 0.001$), regardless of drying method. There was not a significant difference between the pathogen load of post-dry rockweed and dry-stored rockweed because *V. vulnificus* was un-recoverable across all dried samples, regardless of drying method or product type,

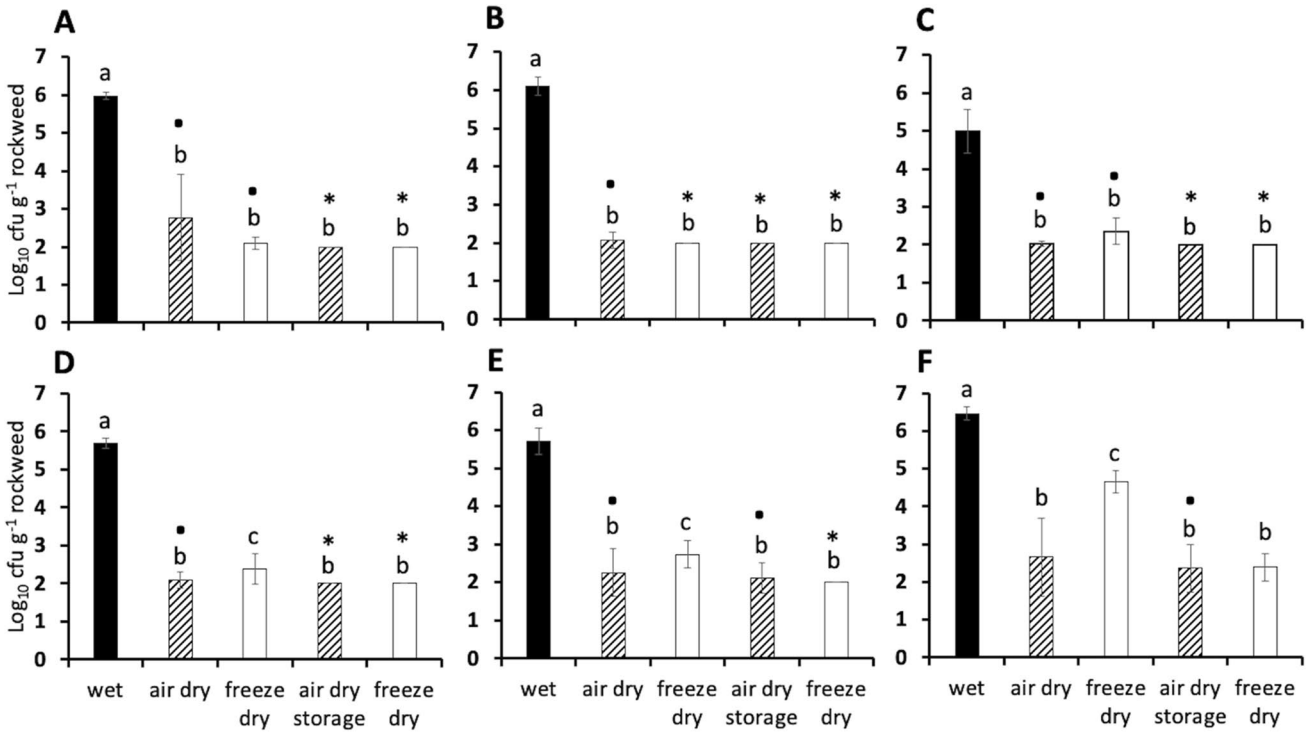

Fig. 5 Effect of drying method on pathogen load of rockweed. Pathogen load was evaluated as the $\log_{10}$ colony forming units per gram of kelp, shown on the y-axis. The product type sampled is shown on the x-axis: "wet" represents kelp sampled before any drying occurred, "air/freeze-dry" represents 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 letters identify significant differences ($p < 0.05$) between treatments. The LOD of our sampling method was log 2 (100 cfu g^{-1}). 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

except for one incidence of low level recovery on a post-dry air-dried sample.

L. monocytogenes Product type ($F_{2,29} = 290.678$, $p < 0.001$) impacted the *L. monocytogenes* population on rockweed, with no effect from drying method ($F_{1,29} = 0.941$, $p = 0.340$) or interaction ($F_{2,29} = 0.941$, $p = 0.402$) (Fig. 5C). This significance was explained by an overall reduction in pathogen load as a result of drying. The *L. monocytogenes* load on wet rockweed was > 2 log higher than on both post-dry ($p < 0.001$) and dry-stored rockweed ($p < 0.001$), regardless of drying method. Although there was no significant difference between the pathogen load of post-dry rockweed and dry-stored rockweed due to our LOD, 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 ($F_{1,63} = 4.595$, $p < 0.05$), product type ($F_{2,63} = 2724.667$, $p < 0.001$) and their interaction ($F_{2,63} = 4.595$, $p < 0.05$) impacted the *E. coli* population on rockweed (Fig. 5, D). This significance was driven by a reduction in pathogen load as a result of drying. The *E. coli* load on wet rockweed was > 3 log higher than on post-dry rockweed that was both air- ($p < 0.001$) and freeze-dried ($p < 0.001$) as well as on dry-stored rockweed that was both air- ($p < 0.001$) and freeze dried ($p < 0.001$). Additionally, for freeze-dried rockweed, 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 ($F_{2,66} = 634.869$, $p < 0.001$) and its interaction with drying method ($F_{2,66} = 3.992$, $p < 0.05$) impacted the *S. enterica* population on rockweed, with no effect from drying method ($F_{1,66} = 1.803$, $p = 0.184$) (Fig. 5E). This significance was driven by a reduction in pathogen load as a result of drying. The *S. enterica* load on wet rockweed was > 2–3 log higher than on post-dry rockweed that was both air- ($p < 0.001$) and freeze-dried ($p < 0.001$) as well as on dry-stored rockweed that was both air- ($p < 0.001$) and freeze dried ($p < 0.001$). Additionally, for freeze-dried rockweed, 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 ($F_{1,28.056} = 13.383$, $p < 0.01$), product type ($F_{2,28.054} = 171.195$, $p < 0.001$) and their interaction ($F_{2,28.054} = 13.118$, $p < 0.001$) impacted the *S. aureus* population on rockweed (Fig. 5F). This significance was driven by a reduction in pathogen load as a result of drying. The *S. aureus* load on wet rockweed was > 1–4 log higher than on post-dry rockweed that was both air- ($p < 0.001$) and freeze-dried ($p < 0.001$) as well as on dry-stored rockweed that was both air- ($p < 0.001$) and freeze dried ($p < 0.001$). Additionally, for freeze-dried rockweed, 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 ($p < 0.001$).

Discussion

Here, we report that storage temperature and drying method had significant impacts on seaweed-associated microbial load. Specifically, 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.

Impact of storage temperature on seaweed pathogen load

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 h 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 (Boye et al. 1999). 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 h. 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 possible that *L. monocytogenes* has a sensitivity to a sugar kelp constituent that was released during its degradation at room temperature (Cox et al. 2010). 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). Additionally, 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 *Enterococcus faecalis* (Cox et al. 2010). Similarly, laminarin extracted from *A. nodosum* was found to effectively inhibited the growth of *S. aureus*, *L. monocytogenes*, *E. coli* and *S. enterica* Typhimurium (Kadam et al. 2015). Innate antimicrobial activity may explain the observed decline of *L. monocytogenes* as well as *V. parahaemolyticus, S. aureus,* and *V. vulnificus* on sugar kelp and *S. enterica*, *E. coli*, *S. aureus*, and *V. vulnificus* on rockweed, which all showed significant reductions in pathogen load for one or all storage temperatures over time.

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.

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. Despite the existence of evidence that all of the pathogens investigated may associate with various species of kelp in either the pre or post-harvest environments, the likelihood of subsequent survival on kelp surfaces seems to vary significantly depending on the kelp species. This finding has significant bearing on the resulting level of practical risk posed by each foodborne pathogen, implying that approaches for maximizing food safety should not be generalized across "seaweeds" as a category of food.

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.

Impact of drying method on seaweed pathogen load

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 by a_w. Kelp dried in this study met the accepted standard a_w to prevent the replication of foodborne pathogens, which is ≤ 0.85 (Beuchat et al. 2011). *S. aureus* is well-documented for its ability to tolerate lower a_w levels than other foodborne 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.

We observed a significantly greater impact of air-drying compared to freeze-drying on *E. coli* and *S. aureus* associated with sugar kelp and *E. coli*, *S. enterica,* and *S. aureus* associated with rockweed. Although freeze-dried products often retain higher quality and nutritional characteristics than food products dried by other means (Bhatta et al. 2020), it is inherently less damaging to microbial cells. Indeed, freeze-drying is often used to preserve bacterial cultures with minimal impact to microbial viability (Morgan and 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 reduction from a natural contamination level of 6.13 log cfu g^{-1}. Similarly, when the effect of freeze-drying on fresh cilantro inoculated with ~ 6 log cfu g^{-1} cocktails of pathogenic *E. coli*, *S. enterica* and *L. monocytogenes* was investigated, 1.5—2 log reductions were found for *E. coli* and *S. enterica*, respectively, and a 0.71 log reduction was found for *L. monocytogenes* (Bourdoux et al. 2018). Though freeze-drying reduces the pathogen load on kelp's surface as effectively as on the surfaces of 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. With that said, the validation of air drying as a "kill step" for processed foods is significantly complex due to the inability to regulate factors such as UV light exposure, humidity and air velocity in an open-air drying system.

It is vital to reiterate that food pathogens are robust and although low a_w can prevent replication it does not necessarily result in microbial death. When under duress, even 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, 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 and 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 challenge tests 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. Repeating this study with a higher inoculum concentration or lower LOD could address this. Additionally, 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 (FAO and WHO 2022). 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 and how it is affected by common processing procedures.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10811-023-02937-w.

Acknowledgements We would like to thank Maine's many edible seaweed industry participants for their input and collaboration. Additionally, we thank Dr. Zachary Olson for his contributions to the statistical analysis of our results. We acknowledge Tim Arienti and Emilly Schutt for field assistance including deployment and maintenance of the sugar kelp farms. This project was made possible with funding from Maine Sea Grant (NA18OAR4170103) and supplemented with support from University of New England College of Arts and Sciences.

Authors' contributions CJB and KMB contributed to the study conception and initial design. Experimental design and presentation of data outputs were informed by discipline expertise of JJP. Material preparation and data collection were performed by JGV, KMB, CM, and LM.

Data and statistical analysis were primarily performed by JGV. The first draft of the manuscript was written primarily by JGV, with KMB, CJB, JJP contributing heavily to edits. All authors read and approved the final manuscript.

Funding This research was supported by the Maine Sea Grant NA18OAR4170103 and the University of New England College of Arts and Sciences.

Data availability The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Competing interests The authors have no competing interests to declare that are relevant to the content of this article.

References

Akomea-Frempong S, Skonberg DI, Camire ME, Perry JJ (2021) Impact of blanching, freezing, and fermentation on physicochemical, microbial, and sensory quality of sugar kelp (*Saccharina latissima*). Foods 10:2258

Allard MW, Muruvanda T, Strain E, Timme R, Luo Y, Wang C, Keys CE Payne J, Cooper T, Luong K, Song Y, Chin C-S, Korlach J, Roberts R, Evans P, Musser SM, Brown EW (2013) Fully assembled genome sequence for *Salmonella enterica* subsp. enterica Serovar Javiana CFSAN001992. Genome Announc 1:e0008113

Bailey TW, do Nascimento NC, Bhunia AK (2017) Genome sequence of *Listeria monocytogenes* strain F4244, a 4b serotype. Genome Announc 5(49):e01324–17

Barberi ON, Byron CJ, Burkholder KM, St Gelais AT, Williams AK (2020) Assessment of bacterial pathogens on edible macroalgae in coastal waters. J Appl Phycol 32:683–696

Beuchat L, Komitopoulou E, Betts R et al (2011) Persistence and survival of pathogens in dry foods and dry food processing environments. ILSI Europe Report Series 2011:1–48

Beuchat LR, Komitopoulou E, Beckers H, Betts RP, Bourdichon F, Fanning S, Joosten HM, Ter Kuile BH (2013) Low–water activity foods: Increased concern as vehicles of foodborne pathogens. J Food Protect 76:150–172

Bhatta S, StevanovicJanezic T, Ratti C (2020) Freeze-drying of plant-based foods. Foods 9:87

Bintsis T (2017) Foodborne pathogens. AIMS Microbiol 3:529–563

Blikra MJ, Løvdal T, Vaka MR, Roiha IS, Lunestad BT, Lindseth C, Skipnes D (2019) Assessment of food quality and microbial safety of brown macroalgae (*Alaria esculenta* and *Saccharina latissima*). J Sci Food Agricult 99:1198–1206

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 CO_2 drying. Innov Food Sci Emerg Technol 47:180–186

Boye K, Høgdall E, Borre M (1999) Identification of bacteria using two degenerate 16S rDNA sequencing primers. Microbiol Res 154:23–26

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. 38 pp

Cox S, Abu-Ghannam N, Gupta S (2010) An assessment of the antioxidant and antimicrobial activity of six species of edible Irish seaweeds. Int Food Res J 17:205–220

Cusson AJ, Burkholder KM, Byron CJ, Grebe GS, Deaveau AM (2021) Impact of time of harvest and drying method on antimicrobial activity of *Saccharina latissima* against two *Staphylococcus aureus* strains. Appl Phycol 2:80–88

Deveau AM, Miller-Hope Z, Lloyd E, Williams BS, Bolduc C, Meader JM, Weiss F, Burkholder KM (2016) Antimicrobial activity of extracts from macroalgae *Ulva lactuca* against clinically important Staphylococci is impacted by lunar phase of macroalgae harvest. Lett Appl Microbiol 62:363–371

FAO and WHO: Food and Agriculture Organization of the United Nations and the World Health Organization (2022) Report of the Expert Meeting on Food Safety for Seaweed - Current Status and Future Perspectives. Rome, 28-29 October 2021. Food Safety and Quality Series No. 13. Rome. https://doi.org/10.4060/cc0846en

FDA: United States Food and Drug Administration (2022) Seafood HACCP. https://www.fda.gov/food/hazard-analysis-critical-control-point-haccp/seafood-haccp. Accessed 11 Apr 2022

Hussain MA, Dawson CO (2013) Economic impact of food safety outbreaks on food businesses. Foods 2:585–589

Kadam SU, O'Donnell CP, Rai DK, Hossain MB, Burgess CM, Walsh D, Tiwari BK (2015) Laminarin from Irish brown seaweeds *Ascophyllum nodosum* and *Laminaria hyperborea*: Ultrasound assisted extraction, characterization and bioactivity. Mar Drugs 13:4270–4280

Lachnit T, Blümel M, Imhoff JF, Wahl M (2009) Specific epibacterial communities on macroalgae: phylogeny matters more than habitat. Aquat Biol 5:181–186

Liu Y, Wikfors GH, Clark P, Pitchford S, Krisak M, Dixon MS, Li Y (2022) A deep dive into the epibiotic communities on aquacultured sugar kelp Saccharina latissima in Southern New England. Algal Res 63:102654

Løvdal T, Lunestad BT, Myrmel M, Rosnes JT, Skipnes D (2021) Microbiological food safety of seaweeds. Foods 10:2719

Lytou AE, Schoina E, Liu Y, Michalek K, Stanley MS, Panagou EZ, Nychas G-JE (2021) Quality and safety assessment of edible seaweeds *Alaria esculenta* and *Saccharina latissima* cultivated in Scotland. Foods 10:2210

ME DMR: Maine Department of Marine Resources (2021) Harvest of Farm-raised Marine Algae in Maine. https://www.maine.gov/dmr/sites/maine.gov.dmr/files/docs/MarineAlgaeTable2015-2021.pdf. Accessed 4 Apr 2022

ME DMR: Maine Department of Marine Resources (2022) Historical Maine Rockweed Landings. https://www.maine.gov/dmr/sites/maine.gov.dmr/files/docs/rockweed.table.pdf. Accessed 4 Apr 2022

Morgan C, Vesey G (2009) Freeze-drying of microorganisms. In: Encyclopedia of Microbiology. 3rd Edn. Academic Press, Oxford pp 162–173

National Sea Grant Law Center (2020) Seaweed Food Safety: Federal Considerations. Recorded informational webinar sponsored by the

National Sea Grant Law Center on 27 April 2020. https://www.youtube.com/watch?v=Ap7J9dtJE4s. Accessed 27 Mar 2023

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 Microbiol 94:103646.

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

R Core Team (2020). https://www.r-project.org/index.html. Accessed 24 Mar 2023

Schmid-Hempel P, Frank SA (2007) Pathogenesis, Virulence, and Infective Dose. PLoS Pathogens 3:e147

Shin LER, Zzaman W, Kuang YT, Bhat R (2015) Influence of dehydration techniques on physicochemical, antioxidant and microbial qualities of *Ipomoea aquatica* Forsk.: An underutilized green leafy vegetable. J Food Process Preservat 39:1118–1124

Silva A, Silva SA, Carpena M, Garcia-Oliveira P, Gullón P, Barroso MF, Prieto MA, Simal-Gandara J (2020) Macroalgae as a source of valuable antimicrobial compounds: Extraction and applications. Antibiotics (Basel) 9:642

Swinscoe I, Oliver DM, Ørnsrud R, Quilliam RS (2020) The microbial safety of seaweed as a feed component for black soldier fly (*Hermetia illucens*) larvae. Food Microbiol 91:103535

Syamaladevi RM, 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. Compr Rev Food Sci Food Safety 15:353–370

US FDA (2018) Background on the FDA Food Safety Modernization Act (FSMA). US Department of Human Health and Human Services / US Food and Drug Administration (FDA). https://www.fda.gov/food/food-safety-modernization-act-fsma/background-fda-food-safety-modernization-act-fsma. Accessed 11 Apr 2022

U.S. FDA: United States Food and Drug Administration (2011) FDA Food Safety Modernization Act 21 U.S.C & 2201. https://www.govinfo.gov/content/pkg/PLAW-111publ353/pdf/PLAW-111publ353.pdf. Accessed 17 Apr 2021

U.S. FDA: United States Food and Drug Administration (2020) National Shellfish Sanitation Program (NSSP). https://www.fda.gov/food/federalstate-food-programs/national-shellfish-sanitation-program-nssp. Accessed 11 Apr 2022

U.S. FDA: United States Food and Drug Administration (2021) 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. Accessed 11 Apr 2022

Wesche AM, Gurtler JB, Marks BP, Ryser ET (2009) Stress, sublethal injury, resuscitation, and virulence of bacterial foodborne pathogens. J Food Protect 72:1121–1138

Wesche AM, Ryser ET (2013) 7. Cell Injury and methods of analysis. In: Salfinger Y, Tortorello ML (eds) Compendium of Methods for the Microbiological Examination of Foods. APHA Press, Washington, DC