Stochastic Modelling and Computational Sciences

FOOD SAFETY OF SEA MOSS

Keshava Reddy Depa

ABSTRACT

The use of sea mosss in the human diet has a long history in Asia and has now been increasing also in the western world. Concurrent with this trend, there is a corresponding increase in cultivation and harvesting for commercial production. Edible sea moss is a heterogenous product category including species within the green, red, and brown macroalgae. Moreover, the species are utilized on their own or in combinatorial food products, eaten fresh or processed by a variety of technologies. The present review summarizes available literature with respect to microbiological food safety and quality of sea moss food products, including processing and other factors controlling these parameters, and emerging trends to improve on the safety, utilization, quality, and storability of sea mosss. The over- or misuse of antimicrobials and the concurrent development of antimicrobial resistance (AMR) in bacteria is a current worldwide health concern. The role of sea mosss in the development of AMR and the spread of antimicrobial resistance genes is an underexplored field of research and is discussed in that context. Legislation and guidelines relevant to edible sea moss are also discussed.

Keywords: sea moss, macroalgae, food safety, microbiology, bacteria, viruses, seafood, foodborne disease, spoilage, food quality.

1. INTRODUCTION

The global sea moss industry is worth more than USD 6 billion per year, corresponding to approx. 12 million tons/year in volume, of which about 85% comprises food products for human consumption (FAO, 2018). Owing to the fact that there will be an increasing need for protein food sources to accommodate the anticipated growth in the world's population, the sea moss industry (both aquaculture and wild-harvested) is expected to grow since sea moss is a sustainable food source. This assumed increase, together with consumers' demands for tasty, nutritious, safe, and convenient sea moss food products, and changes in market trends, leads to a growing need to ensure microbially safe sea moss food products. Several studies have focused on the bacterial diversity in brown (Phaeophyceae), green (Chlorophyta), and red (Rhodophyta) macroalgae (henceforward: sea moss). Bacteria inhabiting sea moss include the Proteobacteria, Actinobacteria, Bacteroidetes (CFB group), Cyanobacteria, Firmicutes, Planctomycetes, Verrumicrobia, Chloroflexi, Deinococcus- Thermus, Fusobacteria, and Tenericutes, with the Gammaproteobacteria as the most common bacterial clade (Singh & Reddy, 2014; Hollants et al., 2013). However, there are only a few studies that specifically clarify the prevalence of human pathogens in edible sea mosss (Hendriksen & Lundsteen, 2014; Lytou et al., 2021).

The total number of bacteria varies according to season and is typically lowest during spring and among younger plants (Mazure & Field, 1980; Lakshmanaperumalsamy & Purushothaman, 1982), but this may also be species- and location- dependent. Although the density and composition of bacteria on sea moss are strongly correlated to that of the surrounding water, it is frequently reported that the microbiota associated with sea moss is different from what is found in the seawater in which they grow (Chan & McManus, 1969; Hollants et al., 2013). A relatively specific bacterial flora can be found to associate with different phyla of marine sea moss growing in the same habitat (Lytou et al., 2021; Kong & Chan, 1979).

The viable counts reach up to log 7 bacterial cells per gram of sea moss biomass when using agar spread plate methods and are shown to be higher when applying direct (microscopy-based) techniques (Table 1).

Table 1. Reported viable bacteria counts for selected sea moss species of relevance for human consumption.

Table 1: Bacterial Density in Sea Moss Species (Wild, Cultivated, or Unknown) by Sampling Location and Method

Sea Moss Species	Wild (w), Cultivated (c), or Unknown (u)	Location/Region	Month and Year of Sampling	Bacterial Density (Log cfu/g)	Method	Reference
Gracillaria spp.	(u)	Mactan Island, Uranouchi Inlet	March, 1996	~8–9	DAPI-staining (M)	Largo et al., 1997
Kappaphycus alvarezii	(c)	Tosa Bay, Southern Japan	Not given	~5	Plate Count (PC), aerobic, 37°C	-
Ulva lactuca	(c)	Izmir, Turkey	March and April	4.94	Plate Count, aerobic, 37°C	Karacalar & Turan, 2008
Palmaria palmata	(w)	County Antrim, Northern Ireland (55°N)	Not given	5.11	Plate Count, aerobic, 30°C	Moore et al., 2002
Caulerpa lentillifera	(c)	Okinawa, Japan	August–September, 2006	Not given	Marine Agar, aerobic, 25°C	-
Gracilaria changii	(c)	Mengabang	December, 2007	8.46	Tryptic Soy Agar with 2% NaCl, room temperature	Musa & Wei, 2008
Alaria esculenta	(c)	West Norway	March and April, 2019	5.2	Marine Agar, aerobic, 25°C	Blikra et al., 2019
Saccharina latissima	(c)	West Norway (61° N)	2016	1.10	Marine Agar, aerobic, 25°C	-
Alaria esculenta	(c)	Port-a-Bhuiltin Sea Moss Farm	2020	3.2	Marine Agar, aerobic, 25°C	Lytou et al., 2021
Saccharina latissima	(c)	Scotland	2019	3.7	Marine Agar, aerobic, 30°C	-
Laminaria hyperborea	(w)	West Norway (60° N)	May, 2007	~6	DAPI-staining (M)	Bengtsson et al., 2010
Fucus serratus	(w)	North-western region	Not given	7.7	Scanning Electron Microscopy (M)	Rogerson, 1991
Porphyra umbilicalis	(w)	Millport, Scotland	July and August, 1990	7.2	Electron Microscopy (M)	-
Palmaria palmata	(u)	Commercial products, Italy	Not given	3.09–5.31	Marine Agar, aerobic, 30°C	Martelli et al., 2021
Laminaria spp.	(u)	Commercial products, Italy	Not given	2.23–4.54	Marine Agar, aerobic, 30°C	-
Ulva spp.	(u)	Commercial products, Italy	Not given	2.88–5.58	Marine Agar, aerobic, 30°C	-
Hizikia fusiformis	(u)	Commercial products, Italy	Not given	2.23–4.35	Marine Agar, aerobic, 30°C	-
Alaria esculenta	(c)	West Norway (61° N)	March, 2015	3.59	Marine Agar, aerobic, 25°C	Unpublished
Laminaria digitata	(c)	West Norway (61° N)	March, 2015	2.79	Marine Agar, aerobic, 25°C	Unpublished
Saccharina latissima	(c)	West Norway (61° N)	February, 2020	3.63	Marine Agar, aerobic, 25°C	-

Notes:

- **PC** refers to Plate Count Agar.
- **M** refers to Microscopy methods such as DAPI-staining or Electron Microscopy.
- **Agar, aerobic, X°C** specifies the agar type and the temperature used for the microbial growth

After the first impression formed by aroma, color, and general appearance, the number of microorganisms on the fresh edible sea mosss may serve as a secondary indicator for the food quality and safety of the edible sea moss, but not more so than for fruits and vegetables, which can have comparable bacterial loads on their surfaces. A high bacterial count of sea moss is indicative of the age and health of the plant, but primarily of the microbial load and composition of the surrounding water masses. High initial bacterial loads normally affect the shelf life and sensorial quality of the product negatively, but do not necessarily imply that the food is unsafe to consume. On the other hand, a low bacterial number does not necessarily imply that it is safe. For some pathogens, especially for the toxin-producing bacteria, consumption of relatively small amounts is sufficient to cause severe health problems in humans, and even death.

There is a general assumption that human pathogens occur on sea moss in the same density and composition as in the surrounding water masses. Hence, the localization of the sea moss is an important factor concerning microbiological food safety (Hendriksen & Lundsteen, 2014; Musa & Wei, 2008; Ziino, Nibali, & Panebianco, 2010; Fødevarestyrelsen, 2021). However, sea moss food products may also get contaminated or re-contaminated during handling and processing (Banach et al. 2020 ; Banach et al., 2020). Locations in coast-near areas with poor water quality may be predisposed to human pathogens. Researchers concluded that consumption of sea mosss collected in Danish waters is safe, as long as harbors and areas influenced by agricultural and industrial run-off are avoided (Martelli et al., 2021). A Norwegian study concluded that—although sea moss is densely covered by bacteria, including potential pathogens that may be challenging during processing or improper storage—the risk of macroalgae as the origin of foodborne diseases cannot be expected higher than for other non-filtering marine organisms, including fish (Duinker et al., 2016).

The increasing use of antimicrobials in, e.g., aquaculture has led to concerns about the development of antimicrobial resistance (AMR) in bacteria and the spread of antibiotic resistance genes (ARG) and that it may compromise successful treatment of bacterial infections (Ferri et al., 2017). The presence of resistant bacteria in the human food supply chain is documented (Bennani et al., 2020), but the role of sea moss is not yet clear. This represents a data gap that warrants more research.

This review is restricted to studies of microbiological food safety of marine sea moss belonging to the brown, green, and red algae. Antimicrobial properties of sea mosss, their derived extracts, or microbial symbionts, are not covered in the present review, nor nutrition or sensory aspects of edible sea moss. The review focuses on human pathogens that may challenge food safety, and not pathogens that may exclusively be detrimental to the plant itself.

2. PATHOGENIC MICROORGANISMS IN SEA MOSS

Bacteria, viruses, yeast, and molds may constitute potential microbiological health hazards in edible sea moss. Regarding bacteria, separation is made between (i) pathogenic bacteria that may be present in such small amounts that it does not lead to a directly observable effect (flavor, color, aroma) of the product, but as by ingestion of minute quantities may still cause food poisoning and even death, and (ii) spoilage bacteria, which is not necessarily harmful to the consumer, but which degrade the product. The main factors for bacterial contamination of seafood are contamination of the raw material from the environment and from the processing, and bacterial growth conditions. The following Section deals with pathogenic microorganisms associated with edible sea mosss (Selvarajan et al., 2019). The specific processing factors that are relevant for sea moss in the frame of food safety and quality, are discussed in more detail in Section 3.

2.1 Bacillus sp.

More than 140 species are at present included in the genus Bacillus (Logan et al., 2009), and they are commonly described as Gram-positive, rod-shaped, straight, or slightly curved cells, that appear singly, in pairs, chains, or as long filaments. They are further referred to as possessing the ability to form resistant endospores, one per cell, although sporulation remains to be documented in some of the recently described species. Bacillus spp. are commonly aerobic, but some species are facultatively anaerobic, and at least two strictly anaerobes have been described. Although the majority of the species belonging to the genus Bacillus have little or no pathogenic potential, some species are known to be associated with food-borne diseases in humans, by means of the production of heat-stable toxins. B. cereus may cause food poisoning and opportunistic infections, while some other species, including B. subtilis, B. pumilus, and B. licheniformis, have also been associated with food poisoning and human/animal infections (Logan et al., 2009; Madslien et al., 2013).

Bacillus spp., among others, are efficient producers of compounds with antibacterial, antifouling, and quorum sensing inhibiting features, which make them highly successful colonizers of sea moss surfaces, and may live in an endosymbiotic relationship with sea- weed (Hollants et al., 2013). Growth promoting and nutritional effects beneficial to the sea moss have been attributed to endophytic Bacillus spp., including B. cereus, B. pumilus, and B. licheniformis, and these species are associated with sea moss of the brown, green and red algae (Jamal et al., 2006; Singh et al., 2011)

Concerns were raised about B. cereus in various dehydrated, ready-to-eat (RTE) sea- weed products sold in Italy (Martelli et al., 2021), B. subtilis on edible brown sea moss harvested off the coast of Ireland (Gupta et al., 2010), Bacillus spp. in sea moss cultivated in Scotland (Lytou et al., 2021), and B. licheniformis and B. pumilus on edible brown sea moss cultivated in Norway (Blikra et al., 2019). Although the concentrations of Bacillus spp. observed on fresh sea mosss may be low compared to what is considered as the infectious dose, measures need to be taken to control the growth of these species in the food during handling and storage (Song et al., 2009). This was demonstrated by a probability distribution model for levels of B. cereus in RTE kimbab (rolled cooked rice and other foodstuffs in dried green sea moss) which estimated that contamination levels at the time of consumption ranged from −3.63 log cfu/g to 7.31 log cfu/g when the model parameters storage time (2.31 ± 4.63 h) and temperature (22.5 ± 3.17 ◦C) (Oh et al., 2004), and conservative initial B. cereus concentrations (−4.85–0.69 log cfu/g [undetectable]) (Park et al., 2005) were based on relevant data surveyed from stores selling RTE kimbab in Korea (Bahk et al., 2007). Kimbab is a RTE type of take-away food that is typically prepared by hand and stored at room temperature, which is probably contributing strongly to contamination and growth It is the Bacillus toxins that are the actual harmful agent, and not the bacteria them- selves, so it is not straightforward to derive a generalized infective dose based on the contamination level. However, for B. cereus, B. pumilus, and B. licheniformis, concen- trations needed to produce enough toxin to induce food poisoning is considered to be ≥log 5 cfu/g (Kramer & Gilbert, 1989; Salkinoja-Salonen et al., 1999; Granum & Braid-Parker, 2000; Granum, 2007). In relation to combinatorial food products with sea moss, as e.g., kimbab, contaminating bacteria (e.g., Bacillus spp. and Staphylococcus aureus), may well originate from e.g., rice or soybean paste, and not the sea moss (Kim et al., 2008).

Spores of Bacillus spp., as exemplified in Figure 1, are very resistant to most external factors and can tolerate temperatures over 100 ◦C combined with pH < 3 for several minutes (Setlow, 2006), but will not be able to reproduce under such conditions. Spores present in the product may on the other hand be able to germinate when the conditions allow and reproduce and eventually produce toxins that may lead to food poisoning and in the worst-case death (Picon et al., 2021). Table 2 summarizes limits for growth in relation to temperature, pH, water activity (aW), and water phase NaCl for some human pathogen spore formers in their vegetative form, in addition to some other potentially harmful bacteria associated with sea moss. The growth rate will decrease with lower temperatures and pH until their minimum limit is reached. A sea moss product may be considered safe to eat as long as pH is below 4.3 when stored at ≤4 ◦C (cf. B. cereus). If the product is to be stored at an elevated temperature, pH needs to be lowered to ≤3.7 (cf. Salmonella). B. licheniformis, B. pumilus, and B.

amyloliquefaciens/subtilis are not able to grow or produce toxins at refrigerated temperatures (Table 2).

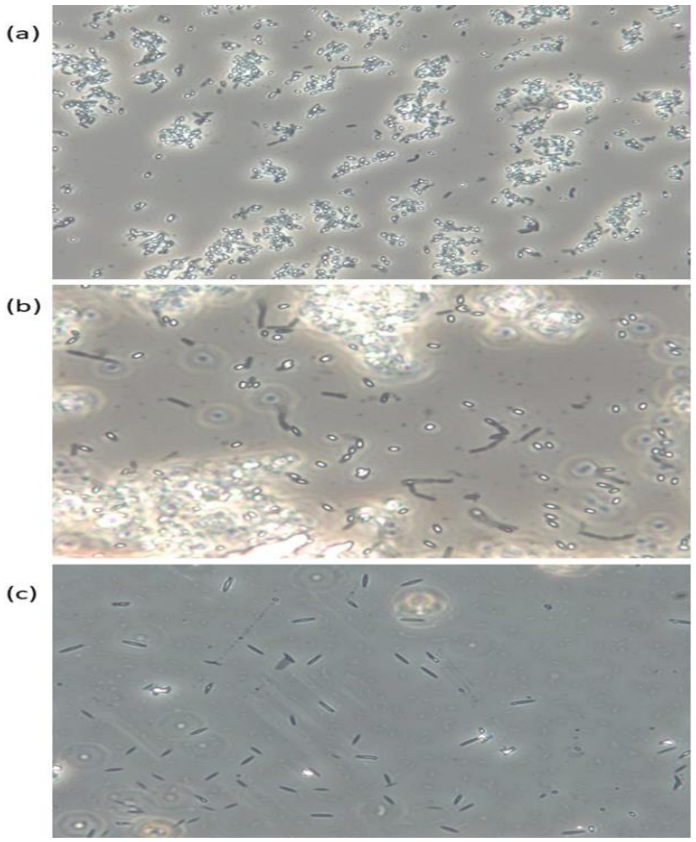

Figure 1. Live phase-contrast microscopy images of (a) *B. licheniformis*, (b) *B. pumilus*, and (c) *B. subtilis* isolated from *Saccharina latissima* and cultivated on Marine Agar. Spores appear white/bright and vegetative cells are dark. Magnification: 400×.

Table 2: Growth Limits for Pathogenic Bacteria of Relevance for Sea Moss

Pathogen	Temperature Min. (°C)	Temperature Max. (°C)	pH Min.	pH Max.	aw Min.	Max. Water Phase NaCl (%)	Reference
Bacillus cereus	4	55	4.3	9.3	0.92	10	U.S. Food and Drug Administration, 1998
Bacillus licheniformis	11–15	50–55	4.6	9.8	0.91	7	Logan et al., 2009; Trunet et al., 2015
Bacillus pumilus	>5–15	40–50	≤6 (Some strains grow at 4.5)	≥9.5	<0.96	>10	From et al., 2007; Samapundo et al., 2014
Bacillus subtilis	5.5	55.7	4.8	9.2	0.93	>5–10	Logan et al., 2009; Gauvry et al., 2021
Clostridium botulinum (proteolytic)	10	48	4.6	9	0.93	10	U.S. Food and Drug Administration, 1998

Clostridium botulinum (non-proteolytic)	3.3	45	5.0	9	0.97	5	U.S. Food and Drug Administration, 1998
Clostridium perfringens	10	52	5	9	0.93	7	-
Pathogenic E. coli	6.5	49.4	4	9	0.95	6.5	U.S. Food and Drug Administration, 1998; International Organization of Standardization, 2009; Clavero & Beuchat, 1996
Listeria monocytogenes	−0.4	45	4.4	9.4	0.92	10	U.S. Food and Drug Administration, 1998
Staphylococcus aureus	Not given	Not given	Not given	Not given	Not given	Not given	-
Salmonella	5.2	42.6	3.7	9.5	0.94	8	U.S. Food and Drug Administration, 1998
Vibrio cholerae	10	~44	5.0	~10	0.97	3	U.S. Food and Drug Administration, 1998; West, Brayton, Bryant, & Colwell, 1986; Adams, Moss, & McClure, 2016
Vibrio parahaemolyticus	5	~44	4.8	~11	0.94	8	U.S. Food and Drug Administration, 1998; West, Brayton, Bryant, & Colwell, 1986; Drake, DePaola, & Jaykus, 2007
Vibrio vulnificus	10	~44	4.4	~9	0.96	6	U.S. Food and Drug Administration, 1998; Drake, DePaola, & Jaykus, 2007; Forsythe, 2010
Aeromonas hydrophila	0	42	6	7.2	0.97	5	U.S. Food and Drug Administration, 1998; Jorgensen, Pfaller, & Carroll

Notes:

- **Temperature Min. / Max.**: Minimum and maximum growth temperatures for the pathogen.
- **pH Min. / Max.**: The pH range for growth of the pathogen.
- **aw Min.**: The minimum water activity level required for growth.
- **Max. Water Phase NaCl (%)**: The maximum percentage of sodium chloride (salt) that allows growth.
- **Reference**: Cited sources for the data.

2.2 Pathogenic Vibrios

Bacteria in the genus Vibrio are Gram-negative, curved rod-formed, and facultative anaerobes (Farmer, 2006). Members of the genus have the sea, brackish and fresh water as their natural habitat and are among the most common bacteria found in surface waters world- wide (Vezzulli et al., 2013). Considering the widespread prevalence of vibrios in aquatic environments, it is not surprising that sea mosss are frequently colonized by members of this genus (Egan et al., 2013). There are currently over 140 Vibrio species, of which 12 are reported to be associated with infections among humans (Bonnin-Jusserand et al., 2019; West, 1989). The most important

human pathogenic species are V. cholerae, V. parahaemolyticus, and V. vulnificus (West, 1989; Baker-Austin et al., 2018), but also several other Vibrio species as V. alginolyticus, V. metschnikovii, V. fluvialis, and V. mimicus may cause infection but with less severe symptoms in humans (West, 1989). The prevalence of human pathogenic vibrios and especially those possessing genes for increased pathogenicity are highly correlated with high water temperatures (Austin, 2010)., and global warming is expected to favor their distribu- tion (Vezzulli et al., 2013). As the vibrios are indigenous to the aquatic environment, there is no documented correlation between the occurrence of Vibrio and commonly applied indicator bacteria of fecal contamination. Thus, indicator organisms as coliforms do not give information on the presence of potentially pathogenic Vibrio spp (Logan & De Vos, 2009).

Water and various foods have been implicated as vehicles for the highly pathogenic V. cholerae O1 and O139 as demonstrated by epidemiologic studies (Centers for Disease Control and Prevention, 2021). A very rare case was reported in which a woman acquired infection after eating raw, fresh sea moss transported from the Philippines as hand luggage to her home in California and eaten a month later (Vugia et al., 1997). However, V. cholera cannot be considered a likely pathogen associated with sea mosss. Food poisoning caused by V. parahemolyticus and V. vulnificus associated with edible sea mosss also appears to be rare, but several documented examples from other kinds of seafood are known, e.g., prawns and oysters (Sumner & Ross, 2002; Honda & Iida, 1993), the latter usually in immunocompro- mised individuals. Findings of V. parahemolyticus (Mahmud et al., 2007). and V. vulnificus (Mahmud et al., 2008) in sea mosss collected along the coast of Japan, prompted the authors to encourage proper hygiene practice during postharvest handling of sea mosss, especially in summer when the con- centrations peaked. Vibrio spp. counts as high as log 8.2 cfu/g have been reported on raw cultivated Gracilaria changii harvested in Malaysia, indicating the potential presence of human pathogens possibly compromising food safety if consumed raw (Musa & Wei, 2008).

The vibrios are considered particularly sensitive to food processing, especially thermal treatment. However, in samples of sundried Ulva lactuca cultivated in Turkey, Vibrio spp. were reported in a number of <10 cfu/g (Karacalar & Turan, 2008). Using sensitive qPCR assays combined with microbial pre-enrichment, Barberi et al., 2020 (Barberi et al., 2020). detected pathogenic V. parahemolyticus in 78% of cultivated sea moss samples from North-East USA. Kudaka et al., 2008 (Kudaka et al., 2008) identified V. parahemolyticus in 18.8% of samples of Caulerpa lentillifera (Sea grape) cultivated in tanks. Although the thermostable hemolysin gene was not detected in any of the isolates, these findings led the authors to highlight the importance of a suitable sterilization process for C. lentillifera to ensure food safety (Kudaka et al., 2008). V. alginolyticus was isolated from cultivated A. esculenta in Scotland, but not V. vulnificus, V. parahemolyticus, or V. cholera (Lytou et al., 2021). Conventional culturing methods failed to identify Vibrio spp. in sea mosss collected in Ireland Moore et al. (2002) or Norway (Blikra et al., 2019).

Ziino et al., 2010 (Ziino et al., 2010) reported a high prevalence (75%) and relatively high densities (log 1.30–4.60 cfu/g) of Vibrio spp. in the traditional sea moss dish “mauro” (i.e., Chondrus crispus and Chondracanthus teedii) sold in Catania, Sicily, Italy, and eaten raw. The most frequently isolated species were V. alginolyticus, followed by V. parahemolyticus, V. coralli- itycus, and V. mimicus, all of which included strains with genomes encoding one or more of the virulence genes ToxR, ToxRS, tlh, or trh. However, of these species, it is only V. parahemolyticus that is considered a food-borne human pathogen. As pointed out by the authors (Ziino et al., 2010), the reason for the high amounts of potential pathogens, in this case, may be that the sea moss was collected in the height of summer in an area used for recreational activities causing anthropogenic contamination, again highlighting the importance of col- lecting sea mosss in unpolluted waters of a high quality. Furthermore, it cannot be ruled out that cross-contamination occurred during handling. Potentially pathogenic Vibrio species have occasionally been detected in the environ- ment and seafood organisms from temperate waters (Håkonsholm et al., 2020), but sea moss has so far not been identified as a challenge regarding vibrios (Duinker et al., 2016).

2.3 Aeromonas sp.

The genus Aeromonas belongs to the family Aeromonadaceae, and is a group of Gram-negative, rod-shaped, oxidase- and catalase-positive and facultatively anaerobic bacteria (Colwell et al., 1986; Martin-Carnahan & Joseph, 2005). Members of this genus are ubiquitous aquatic bacteria and thus common in environments such as fresh-, brackish and marine water, and also found as inhabi- tants of aquatic animals (Martin-Carnahan & Joseph, 2005). Aeromonas spp. are potential foodborne pathogens and known to cause gastrointestinal as well as extra-intestinal infections in humans (Tomás, 2012). Most studies have dealt with A. hydrofila, which have been implicated in many seafood-borne outbreaks (Sheng & Wang, 2021). The occurrence of Aeromonas spp. has been frequently reported in water and food, including RTE seafood [(Di Pinto et al., 2012; Lee et al., 2021). Currently not much is known on the role of sea mosss as responsible food for infections. However, based on their indigenous aquatic prevalence, Aeromonas spp. could be expected to colonize sea mosss and possibly follow the raw materials to processing. Furthermore, the ability of some Aeromonas sp. to survive and even grow at chilled temperatures gives reason for concern for sea moss and other seafood products. A. hydrophila was isolated from e.g., Ulva reticulata harvested in Malaysia (Vairappan & Suzuki, 2000), and Aeromonas spp., in concentrations up to log 5.9 cfu/g, from mauro prepared from Chondrus crispus and Chondracanthus teedii sold by fishmongers or from street stalls in Sicily, Italy (Ziino et al., 2010)

2.4 Escherichia coli, Salmonella spp., Listeria monocytogenes, Staphylococcus aureus, and Other Microorganisms Associated with Health Hazard in Sea moss

Bacterial pathogens on sea mosss for human consumption may origin from two main sources; the environment in which they are grown and from equipment and humans who handle the algae after harvest. Pathogens from environmental and anthropogenic sources may persist in coastal waters and can potentially cause contamination. Research on bacterial pathogen contamination of sea mosss is limited, and literature is scarce for some areas e.g., US coastal waters (Barberi et al., 2020). while more literature is found from other parts of the world. Sugar kelp Saccharina latissima and adjacent water were sampled from three sites of sea moss aquaculture located in adjacent bays of Maine, USA, during the winter growing season (Barberi et al., 2020). Membrane filtration onto selective media detected E. coli and Vibrio species in sea moss and water samples at all sites, however with very low plate counts. The foodborne pathogens Salmonella enterica ser. Typhimurium and enterohemorrhagic E. coli O157:H7 were detected on enriched sea moss samples from 83%, 78%, and 56% of sampling events, respectively, using molecular methods (Barberi et al., 2020).

The Ministry of Environment and Food of Denmark proposed a guideline of 100 cfu/100 g of sea mosss for E. coli, as an indicator organism for fecal pollution, and a limit of none detected in 25 g for Salmonella (Martelli et al., 2021). The hygienic quality of edible sea mosss in Danish waters was assessed by analyzing 65 samples of brown (Fucus vesiculosus, Fucus serratus, Fucus spiralis) and green (Ulva lactuca, and Cladophora spp.) sea mosss distributed along the Danish coastline. The E. coli counts were above the proposed limit in eight samples of the brown sea moss F. vesiculosus, including two samples with >1000 and >3000 cfu/g, respectively, collected in proximity to agricultural run-off or harbor basins. E. coli in the remaining six samples served as a reminder of fecal pollution and possible association with norovirus (Martelli et al., 2021). Salmonella sp. was not detected in any of the 65 samples, prompting the conclusion that, as long as pollution sources and industrial run-off and harbors are avoided, it is safe to collect sea mosss for human consumption in Denmark, but it could not be concluded from the results where, geographically, it is safe (Martelli et al., 2021).

A few European studies failed to detect gastrointestinal pathogens on wild-collected sea mosss, including Laminaria (Blanch et al., 2000; Christy et al., 2004; Tay et al., 2002; Remaud et al., 2018). In a study on Saccharina latissima and Alaria esculenta farmed in Norway, no enterococci, coliforms, pathogenic Vibrio, or Listeria mono- cytogenes were found through plating methods (Blikra et al., 2019). Salmonella, E. coli, and S. aureus were absent in samples of cultivated S. latissima and A. esculenta collected in Scotland, but one sample of A. esculenta was positive for L. monocytogenes, probably as a result of cross-contamination during handling (Lytou et al., 2021).

When analyzing RTE products that include sea mosss, the sources of contamination are more unknown and may have a cause in the failures of hygiene procedures. Cho et al., 2008 (Cho et al., 2008) examined 30 kimbab samples using a multiplex PCR method and found 83.3% of samples contaminated. The contamination rates were for S. aureus (56.7%), B. cereus (43.3%), Salmonella spp. (36.7%), Shigella spp. (13.3%) and L. monocytogenes (6.7%). An examination of 258 kimbab and lunch boxes showed 13.2% contamination and S. aureus, B. cereus, and Yersinia enterocolitica were identified (Kim et al., 2008). S. aureus is frequently found in kimbab in concentrations up to 3.5 log cfu/g (Kim et al., 2008; Park et al., 2005; Cho et al., 2008). In risk assessments of S. aureus for kimbab, a maximum storage time of 5–7 h at ambient temperatures is recommended, dependant on initial numbers of S. aureus, time-temperature relationship, and other growth factors (Rho & Schaffner, 2007) Besides the bacteria mentioned above, the following microorganisms are associated with health hazards in sea moss: Campylobacter jejuni and Yersinia enterocolitica can be isolated from water and seafood but are not reported as a serious health hazard in edible sea moss. The former is very sensitive to NaCl and other environmental factors, and it is mostly non-pathogenic strains of the latter that are isolated from the environment. Y. enterocolitica was detected in less than 1% of kimbab samples, and C. jejuni was not detected in any samples (Kim et al., 2008). It is rare reported outbreaks of seafood-related yersiniosis (Ahmed, 1991). However, if Y. enterocolitica was to contaminate sea moss food products, it is likely that it could grow under refrigeration (Gill & Reichel, 1989) Clostridium spp. was detected in 8.4% of semi-processed or final seasoned roasted laver collected in processing plants in Korea, but not C. botulinum or C. perfringens (Choi et al., 2014). C. perfringens could not be detected in any out of 258 kimbab samples purchased in Korea (Kim et al., 2008). Shigella spp. (S. flexneri and S. sonnei) was found in 13.3% of kimbab samples purchased from supermarkets and convenient stores in Korea using a very sensitive method employing enrichment culture prior to PCR (Cho et al., 2008). Yeasts and molds were not detected in fresh wild Palmaria palmata collected in Northern Ireland Moore et al. (2002), nor in P. palmata or Ulva rigida collected in France (Liot et al., 1993). In air-dried samples of P. palmata harvested in France, some molds (log 2.7 cfu/g) were found after 126 days of storage in the dark at 12 ◦C in sealed (not vacuumed) polyethylene bags (Stevant et al., 2020). The populations of molds/yeast in commercial dried sea moss stored at a relative humidity (RH) of 90% and at 25 ◦C for 15 days were log 6.42 cfu/g, but significantly lower when stored at RH 70% (log 2.12 cfu/g) and 50% (log 1.35 cfu/g) (Hyun et al., 2018). Few international standards specify limits for molds and yeast in sea moss products, except for China. According to the General Administration of Quality Supervision, Inspection, and Quarantine in China (AQSIQ), molds must be <300 cfu/g in dried laver products, to ensure food safety [(Choi et al., 2014; AQSIQ, 2005; AQSIQ, 2009)].

2.5 Viruses

Viruses are intracellular obligate parasites, which means they cannot replicate in the environment outside a cell. Although viruses do not multiply in water or in food matrixes, many viruses still pose a risk as food-borne pathogens (Bosch et al., 2018) due to their low infectious dose and robust survival in the environment (Rzezutka & Cook, 2004). Any virus that is shed in feces can potentially transmit via food, but among registered foodborne viruses that cause disease, norovirus (NV) and hepatitis A virus (HAV) are dominating. Norovirus and HAV are responsible for an estimated 20% and 2% of global foodborne illnesses, respectively (Bosch et al., 2018; CDC, 2021a; CDC, 2021b). Both NV (Caliciviridae) and HAV (Picornaviridae) are small, non-enveloped viruses that contain a single stranded RNA as genomes. Noroviruses constitute several genogroups and genotypes and have a broad animal host range but are not considered zoonotic agents. The human NVs are found in genogroup I and II. Hepatitis A virus is only found in the human intestine and the source of foodborne NV and HAV is, therefore, human feces that contaminates through irrigation water, sewage, surfaces, and handling of food. As non- filter feeders, sea moss may not be considered high risk for food-borne viral transmission compared to e.g., oysters. Histo blood group antigens (HBGA) are cellular intestinal carbohydrate receptors for NV and are also found in oysters (Tian et al., 2007) and on some leafy greens (Esseili et al., 2019), These products are often connected with outbreaks of NV disease, probably due to the binding of NV to the HBGA. Whether these receptors could also be present on seagrass is not known. However, the disease caused by NV has been linked to sea moss. In 2017, more than 2000 persons in Japan got ill with NV gastroenteritis from eating dried shredded sea moss (nori) (Sakon et

al., 2018; Kusumi et al., 2017). The nori was used as a topping on cooked rice. Investigators suspected contamination of sea moss during the shredding process. The processing company stated that the sea moss had been heat-treated at 240 ◦C for seven seconds and subsequently submersed in 90 ◦C water for 2 h but had been handled with bare hands by an infected operator during the subsequent cutting and processing operations (Bai et al., 2020). The epidemiologic studies showed that NV maintained infectivity for more than 2 months under dry and ambient temperature conditions. In South Korea, 91 students at two schools got NV disease after consumption of uncooked, vinegar seasoned green sea moss (Park et al., 2015). Vinegar can eliminate some microbes, but NV is resistant to harsh environmental conditions and can remain stable under low pH (Donaldson et al., 2008; Lopman et al., 2012). Investigation of the two outbreaks did not conclude whether the sea moss was contaminated during farming or subsequent washing processes. Further, sea moss imported from China has caused outbreaks in European countries (Whitworth, 2019). In Norway, more than 100 people became ill with NV from imported frozen Wakame sea moss served in restaurants. Norovirus was detected both in patient stool and in the sea moss. Outbreaks in several other European countries were probably linked to this product (Whitworth, 2019). Farming of sea moss in sewage-contaminated water and handling of the sea moss are probably the main routes of viral contamination. Thermal processing is an effective strategy in inactivating foodborne viruses and temperatures ≥90 ◦C for >90 s are generally effective (Bosch et al., 2018). Properly heated sea moss should, therefore, constitute no risk as a vector for infectious enteric viruses, unless the product is contaminated after this process. On the other hand, viruses remain relatively stable under refrigerated and freezing conditions (Bosch et al., 2018).

2.6 Antimicrobial Resistance

Antimicrobial resistance (AMR) is a current worldwide public health concern, where the over- or misuse of antimicrobials in any setting, aquaculture, agriculture, or human medicine, can compromise the successful treatment of bacterial infections (Ferri et al., 2017). Many antibiotic resistance genes (ARGs) originate from natural environments (Martinez, 2008), and environ- ments influenced by anthropogenic activities as waste water discharge and run-off from agricultural land fertilized by animal manure, are considered hotspots for the development and spread of AMR (Berendonk et al., 2015). Bacteria carrying resistance genes can be transmitted between humans, animals, and the environment, including the marine setting (Amarasiri et al., 2020). Even though the marine environment has been characterized as a vast reservoir of ARG (Hatosy & Martiny, 2015), its role in the development and dissemination of AMR to humans is not well understood. Thus, the literature is scarce on AMR in human pathogens in the marine environment, although previous studies have reported resistance among *E. coli*, members of the genus *Vibrio*, and *Klebsiella* sp. (Håkonsholm et al., 2020a, 2020b; Grevskott et al., 2017).

Lately, increased awareness of food as a carrier of AMR and ARG has been seen (Canica et al., 2019; Bergspica et al., 2020). The presence of resistant bacteria is documented in the human food supply chain, which represents a potential exposure route and risk to public health (Bennani et al., 2020) Sea mosss can be involved in AMR development and spread by several mechanisms. The first is the selection of AMR bacteria in the environment by antimicrobial products from sea mosss (Morcom, 2018). Secondly, the conditions on the surface of sea mosss provide a sta- ble environment with a high density of bacteria favoring horizontal genetic transfer of ARG (Morcom, 2018). Finally, sea moss can be contaminated by AMR bacteria during harvest, trans- port, or processing and find their way to the consumer, particularly during consumption in a raw or lightly preserved state. The relative importance of sea mosss in the possible development and spread of AMR in the environment or as food is by far well described, and further study would be needed (Nayyar & Skonberg, 2019).

3. PROCESSING AND FACTORS THAT CONTROL MICROBIAL GROWTH IN SEA MOSS

Processing methods for preservation are intended to make food edible, palatable, and safe so that it can be used beyond the harvest season. According to the FAO Globefish Research Programme (FAO, 2018), dried sea moss products are today totally dominating the market. However, sea mosss have recently become more widespread in new markets and introduced as an ingredient in a number of new products in the US and European market, and

these alternative methods to drying are gaining interest. With the use of sea mosss distributed as raw (fresh or frozen) or minimally processed and intended as an ingredient by the food industry rather than the end, the consumer comes a need for more knowledge on processing. Still, the enhancement of drying technologies due to the increasing focus on sustainable production is of major importance and the food safety aspects must be considered in this perspective.

3.1 Drying

Drying may inhibit all microbial growth including yeast and mold by reducing the water activity (a_W) to 0.6 or below, while bacteria of relevance are inhibited at much higher a_W according to Table 2. The optimal a_W for a food product is usually a compromise between several priorities. At a_W below 0.30, lipid oxidation will occur and Maillard reaction has an optimum at a_W = 0.65 (Mathlouthi, 2001) and high-temperature drying should therefore not be used down to this level. Sea moss processors will, in general, avoid drying to lower moisture content than needed for the preservation of the products as the weight loss and drying costs represent a direct economic loss. Determination of the optimal a_W and moisture content is therefore essential. To achieve this, the relationship between the moisture content of the sea mosss and a_W has to be determined but literature on this has not been found. Some correlations have been documented for other foods, e.g., algae and fish by the method of da Silva et al. (Da Silva et al., 2008). A more fundamental understanding of the relation of water content, a_W, and water structure in foods has been presented by Mathlouthi, 2001 (Mathlouthi, 2001) who proposed a method for determining the correlations and validated it for sugars.

The surface-to-volume ratio is very high for most sea mosss and the drying time is relatively short which makes it feasible to dry at low temperatures (<< 60 ◦C) without risking microbial growth during drying. Typical low-temperature drying methods are sun drying and drying with dehumidified air but may also be achieved by electromagnetic drying by microwaves or radio frequency. The latter may also be used for high-temperature drying alone or in combination with hot air drying, infrared drying, or alternatively by superheated steam drying. These high-temperature drying methods may be designed to inactivate both bacteria and spores of bacteria. This may be of interest when the dried sea mosss are intended for use as ingredients in moist foods intended to have a shelf life after the addition of the sea mosss.

3.2 Thermal Processing

Blanching and boiling of sea mosss are done for several purposes including the inacti- vation of microorganisms and inactivating inherent enzymes causing the breakdown of the product. Brown sea mosss commonly have an unacceptable high concentration of iodine which may be reduced by up to 94% by boiling for a few minutes. However, boiling causes loss of flavonoids and water-soluble nutrients which limits the prevalence (Ho & Redan, 2020). There are currently few thermally processed sea moss products in the market com- pared to dried sea moss, but they are found as ingredients in canned (e.g., mackerel in tomato sauce), pasteurized (e.g., fish burgers), fried and boiled (e.g., soup) products (Kanagasabhapathy et al., 2009).

The edible sea moss laver (*Porphyra umbilicalis*), commonly named nori, is cultivated and consumed in East Asia (Lee, 2010) and is one of the most commonly used sea mosss for human consumption. It is manufactured as dried and/or processed products and is in great demand as side dishes and snacks. Dried laver may be a contamination source to kimbab and in rolled sushi (Kim et al., 2011)., but Choi et al., 2014 (Choi et al., 2014) showed that heat-processed laver (260 to 400 ◦C, 2 to 10 s) had reduced aerobic bacterial counts, and no non-spore-forming pathogens (coliforms, *L. monocytogenes*, *S. aureus*, *Salmonella* spp. and *V. parahaemolyticus*).

Thermoresistant *B. cereus* was occasionally found and suggested as a target organism in the risk assessment. From the heat treatments in the study of Blikra et al., 2019 (Blikra et al., 2019), they also suggested the need to control the growth of toxin-producing spore-forming bacteria such as *B. licheniformis* and *B. pumilus* during handling and storage. The heat inactivation kinetics of *B. cereus* is well described for several growth media but not specifically for sea mosss. The decimal reduction time at 95 ◦C is typically found to be around 10 min or higher for *B. cereus* in agar Fernandez et al. (1999). These values are not necessarily of relevance to sea mosss, as

only less heat-stable spore forms have been documented so far. Gupta et al., 2010 (Gupta et al., 2010) found that heat treatment of 85 ◦C for 15 min inactivated all microorganisms except spore formers which germinated after this treatment and resulted in bacterial counts as high as log 7 cfu/g. They further reported that heat treatment of 95 ◦C for 15 min inactivated all surface microflora (Cundell et al., 1977).

Sea mosss have a low thermal conductivity compared to fish and the leaves may clump together in many layers, resulting in a configuration where it is hard to predict the exact heat load and therefore the heat inactivation of microorganisms may be difficult to assess as well. A popular method of boiling and at the same time increasing the shelf life is vacuum packaging in a sealed pouch or container before the heat treatment, but this can also be challenging. Akomea-Frempong et al., 2021 (Akomea-Frempong et al., 2021) vacuum-packed sugar kelp in bags of 350 g and blanched at 100 ◦C for 3 min and found no significant impact of the heat treatment with respect to the microflora, possibly because of poor heat penetration. The vacuum packaging of thin leaves is challenging, and residual air may be observed. Residual air in pouches may lead to poor heat transfer and cold spots (Skipnes et al., 2002) where microorganisms may survive. Due to the aforementioned information, it is crucial to both perform heat penetration measurements and demonstrate the heat inactivation of a selected target organism by challenge testing.

3.3 Fermentation

Successful fermentation stabilizes the raw sea moss biomass by producing lactic acid and quickly reducing the pH of the sea mosss to below 4.3, where most potentially pathogenic bacteria are inactivated at refrigeration temperatures (pH 3.7 for ambient tem- peratures, cf. Table 2). Lactic acid fermentation of sea moss is a recent strategy and quite limited information is available on culture conditions [(Uchida et al., 2007; Skonberg et al., 2021). The absence of natural lactic acid bacteria (LAB) microflora and simple sugars in most sea mosss, as opposed to terrestrial plants, may have limited development of this technique in the former (Skonberg et al., 2021). Fermentation may be a preferred processing technique for sea mosss because several sea- weed species are sensitive to both thermal treatment and freezing that often diminishes the sensorial properties, appearance, and nutritional value of the products. However, as shown by Uchida et al., 2007 (Uchida et al., 2007), LAB fermentation of *Undaria pinnatifida* is not straightforward due to the selective survival of potential pathogenic spore-forming *Bacillus* spp. through the drying process that could not be effectively outcompeted by the LAB starter culture during fermentation. When cultivated sea moss was mixed with sauerkraut at a ratio of up to 1:1, LAB fermentation proved successful by resulting in sufficiently low pH and thus maintained acceptable microbial and sensorial quality up to 60 days post-inoculation (Skonberg et al., 2021). Heat treatment (95 ◦C for 15 min) followed by fermentation using a commercial *Lactobacillus plantarum* starter culture led to a drop in pH and stabilization at pH 4.5 after 40 h in *Saccha- rina latissima* (Bruhn et al., 2019), and although this is above the limit set at 4.3 in regards to the growth of *B. cereus* (Table 2), no colonies with the morphology of *B. cereus* were observed (Bruhn et al., 2019).

3.4 Freezing

During the freezing of sea mosss, most of the water content is immobilized around the freezing point of seawater which depends on the salt content of the actual sea moss, usually between 0 ◦C and −2 ◦C. Water bound to other molecules has shown a freezing depression in the range −12 ◦C to −25 ◦C before rinsing, but after proper rinsing and loss of salts, the freezing point is increased to 0 ◦C (Tolstorebrov et al., 2019). This change in the freezing point is important for the availability of water to microorganisms.

There is surprisingly little literature available on the freezing of sea mosss, possibly due to the limited changes during long-time frozen storage. Del Olmo, Pico, and Nunez, 2019 (Del Olmo et al., 2019) documented 72% retention of polyphenols and 79% retention of antioxidant capacity after 180 days of storage at −24 ◦C. While freezing to a temperature below −25 ◦C is an effective measure to protect against microbial growth during storage, the damage to the cell structure during freezing and thawing may make the plant more accessible to microorganisms after thawing. During thawing, the drip loss released from the sea mosss may provide a pathway for the microorganisms.

Rapid freezing and thawing are recommended to minimize the risk of microbial growth as well as to limit the drip loss as much as possible. This may be achieved by thin layer band freezers or in vertical plate freezers if the width of the blocks is limited to keep the freezing time below a few hours. Block freezing on racks without air circulation and other methods needing several days to freeze the product will be less effective than rapid freezing with respect to food safety

4. GUIDELINES AND LEGISLATION

The Centre d'Etude et de Valorization des Algues (CEVA) recommended guidelines regarding quantitative limits in dry edible sea moss products, and quantitative limits for sea moss are also introduced in e.g., Korea and China (Table 3). The general principles and requirements of sea moss food safety in the EU are subject to the EU enforced Regulation (EC) no 852/2004 on food hygiene. In many countries, the food manufacturing process is subject to Hazard Analysis and Critical Control Point (HACCP) assessment; a system adopted by the World Health Organization and the Codex Alimentarius Commission as recommended international code of practice for general principles of food hygiene. However, considering the new market trends and novel processing technologies and sea moss food products, guidelines and legislation on specific sea moss food products are still lacking. It is also doubtful whether legislation from one part of the world can be transferred to other areas as well without taking, e.g., biological (sea moss and microbial flora) and environmental (climatic) factors into account

Table 3: Selected standards for microbial load in sea moss food products.

Pathogen	Limit (cfu per g)	Comment Reference
Aerobe mesophiles	≤105	
Coliforms (fecal)	≤10	
Anaerobe sulfite reducers	≤102	French guidelines that apply to dry (CEVA, 2014)]
S. aureus	≤102	sea moss products
C. perfringens	≤1	
Salmonella	Not present per 25 g	
S. aureus	<102	
B. cereus	<103	
Salmonella spp.	0	Korean legislation that applies to RTE (Cho et al., 2008; KFDA, 2008)] foods, including RTE sea moss
Shigella spp.	0	
L. monocytogenes	0	
Aerobic plate counts	<3 × 104 cfu/g	
Coliforms	<30 MPN/100 g	
Mold	<300 cfu/g	Chinese hygienic standard for marine
Salmonella spp.	0	algae and algae products. Applies also (Choi et al., 2014; AQSIQ, 2005; AQSIQ, 2009)
Shigella spp.	0	to dried laver
V. parahemolyticus	0	
S. aureus	0	
E. coli	<100 cfu/100 g	Guidelines for sea moss collected in (Martelli et al., 2021)
Salmonella	Not present per 25 g	Danish waters

3. DATA GAPS

Increased interest in sustainable sea moss diets has opened new markets and applications, necessitating a shift in research focus from traditionally dominating drying processes to novel methods for processing and utilizing sea moss raw materials under bioeconomic principles. For example, systematic and published trials on the preservation of sea mosses through fermentation are relatively scarce, emphasizing the need for further studies on optimal process conditions and their effects on pathogenic bacteria and shelf life (Gill, 2018). Data from Asia on sea moss food safety is abundant, and Europe and the Americas are catching up on research interest concurrent with the market trends and increased consumer demand for sea moss food products. Data from Africa are however scarce, indicative perhaps of the historical and current low levels of commercial interest or value (FAO, 2018)

Sea mosss are densely populated by bacteria on their surfaces, and horizontal gene transfer could occur enhancing the distribution of ARGs. The possible role of sea mosss in the development and spread of AMR in the environment or as food is, by far, well described, and further study would be needed. Predictive microbiology deals with the study of models for microbial growth and survival under particular environmental conditions and it has been developed and im- plemented to predict the occurrence and growth of food-borne pathogens 143. (Kumar,2019). Relatively few predictions are so far carried out for pathogenic bacteria in sea mosss and may reflect lacking data on the support of growth conditions in sea mosss. An exception is modeling on Staphylococcus sp.

4. CONCLUSIONS

The present review has identified pathogenic Bacillus spp., Vibrio spp., and Aeromonas spp. as the main inherent bacteria that are of special concern for the food safety of sea mosss. Bacillus spp. forms heat-resistant spores and can produce heat-stable toxins, whereas Vibrio and Aeromonas spp. can grow under chilled temperatures. Several bacterial species, including E. coli, Salmonella spp., S. aureus, and L. monocytogenes, and Norovirus and Hepatitis A virus, are considered as potential food safety concerns, predominantly by virtue of recontamination during processing. Some other pathogenic bacteria, e.g., Campylobacter spp., Clostridium spp., Shigella spp., and yeast and molds, are considered as sea moss associated and can on rare occasions lead to food poisoning, however presumably because of gross violations of food safety protocol. Further studies and risk analysis, and updated guidelines concerning food safety of both wild-harvested and cultivated sea moss, are necessary. Several preservation technologies are available, but traditional technologies like drying, freezing, and heat treatments, like blanching and pasteurization are still the most obvious ways to achieve food safety. However, due to the energy demands, these processes will continue to be challenged by novel methods. In Asia, where sea mosss have historically been a more important part of the everyday cuisine than in many western countries, expertise on sea moss food preparation and processing has accumulated for generations, and the legislative framework for food safety may have been better incorporated to also include sea moss. Exchange of experiences between East and West will certainly lead to increased knowledge and improved food safety for the benefit of society and consumers. However, biological (sea moss and microbial flora), environmental (climatic), and cultural differences must be accounted for

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