Intervention Strategies for Reducing *Vibrio Parahaemolyticus* in Seafood: A Review

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Abstract: *Vibrio parahaeomolyticus*, a natural inhabitant in estuarine marine water, has been frequently isolated from seafood. It has been recognized as the leading causative agent for seafoodborne illness all over the world. Numerous physical, chemical, and biological intervention methods for reducing *V. parahaeomolyticus* in seafood products have been investigated and practiced. Each intervention method has distinct advantages and disadvantages depending on the processing needs and consumer preference. This review provides a comprehensive overview of various intervention strategies for reducing *V. parahaeomolyticus* in seafood with an emphasis on the efficiency of bacterial inactivation treatments and the changes in sensory qualities of seafood. In the meantime, reported researches on alternative technologies which have shown effectiveness to inactivate *V. parahaemolyticus* in seawater and other food products, but not directly in seafood are also included. The successful applications of appropriate intervention strategies could effectively reduce or eliminate the contamination of *V. parahaeomolyticus* in seafood, and consequently contribute to the improvement of seafood safety and the reduction of public health risk.

Keywords: food safety, intervention method, seafood, *Vibrio parahaeomolyticus*

Introduction

Seafood is nutritious and constitutes an important part of the human diet. According to the Food and Agricultural Organization's (FAO) FAOSTAT database (http://faostat.fao.org), the total seafood supply from the aquaculture production has steadily increased during the past decades in the world. However, seafood is an important vehicle for pathogenic microorganisms. For example, among the 944 *Vibrio* infections occurred in the United States in 2012, 211 patients were reported eating a single seafood item and 53 handling seafood (CDC 2014). As investigated by Feldhusen (2000), at least 10 genera of bacterial pathogens have been implicated in seafoodborne diseases, including *Vibrio spp.*, *Listeria monocytogenes*, *Clostridium botulinum*, *Aeromonas spp.*, *Salmonella spp.*, and *Escherichia coli* O157:H7, among which *Vibrio spp*. were considered as the predominant risk agent. *Vibrios* belong to the family *Vibrionaceae*, which contains 117 recognized species at the time of writing (Euzéby 2014), and at least 13 of them are pathogenic to humans, including the notorious *Vibrio parahaeomolyticus* (VP) (Drake and others 2007).

VP is a halophilic Gram-negative, flagellate, rod-shaped or curved bacterium that prefers to live in an optimum NaCl concentration of 2.5% to 3%, and a warm temperature range of 30 to 35 °C (Kaneko and Colwell 1973; Baumann and Schubert 1984). This microorganism is widely distributed in the marine environments and frequently isolated from a variety of seafood. As shown in Table 1, the incidences of VP in raw, processed, and ready-to-eat seafood products were reported all over the world. Consumption of raw or undercooked seafood contaminated with VP strains carrying either *tdh* or *trh* gene, or both, may lead to acute gastroenteritis characterized by diarrhea, headache, vomiting, nausea, and low fever (Yang and others 2009; Iwahori and Yamamoto 2010). VP was first recognized as the causative agent for seafoodborne illnesses in Osaka, Japan in 1950, with 272 illnesses and 20 deaths caused by consumption of sardines (Fujino 1974). Since then, the bacterium has been reported as the leading cause of seafood poisoning throughout the world. VP accounted for 31.1% of 5770 foodborne outbreaks during 1991 to 2001 in the mainland of China (Liu and others 2004) and 63.8% of foodborne outbreaks between 1995 and 1999 in Taiwan (Chiou and others 2000). In addition, seafoodborne outbreaks associated with VP also occurred in the United States (Iwamoto and others 2010) and European countries (Feldhusen 2000). The high prevalence of VP in seafood presents a great threat to human health.

Several risk assessment studies on VP in seafood products have been conducted. For instance, the Food and Drug Administration (FDA 2005) carried out a risk assessment of VP in raw oyster and suggested that risk per annum (predicted number of illnesses each year) in the United States was 2826. In that study, the effectiveness of several potential inactivation strategies, such as mild heat treatment, irradiation, and freezing was evaluated in several "what-if" scenarios and the results indicated reducing the bacterial levels in oysters by 4.5 log CFU/g by implementing certain methods would reduce the predicted number of illnesses to less than one case per year. Yamamoto and Iwahori (2008) estimated the mean of the expected number of times a person would get ill with VP from consuming bloody clams in Thailand was 5.6×10^{-4}, or approximately 6 in 10000/person/year, and figured out that boiling the clams properly could be the primary method to reduce the risk. Iwahori and Yamamoto (2010) evaluated the risk of consuming raw horse mackerel in Japan and found that the best-case scenario would give a mean probability of illness of 5.6×10^{-6} per meal. Furthermore, the report presented that no wash at landing and exposure to higher temperature before preparation would increase the risk by 7% and 50%, respectively. Consequently, effective microbial inactivation methods should be employed in seafood postharvest processing to reduce seafood illness caused by VP. The

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Table 1–Examples of the incidence of *V. parahaeomolyticus* in seafood around the world.

Country	Seafood	Incidence (%) (No. of positive samples/No. of samples)	Reference
New Zealand	Pacific oyster	94.8 (55/58)	Kirs and others, 2011
Portugal	Fish	35 (7/20)	Davies and others, 2001
Greece	Fish	14 (14/101)	
Italian	Shellfish	32.6 (47/144)	Pinto and others, 2008
China	Fish	32.63 (47/144)	Zhang and others, 2007
	Shrimp	52.76 (67/127)	
	Shellfish	63.33 (19/30)	
United States	Alabama oyster	100 (16/16)	Zimmerman and others, 2007
Japan	Hen clam	94.7 (72/76)	Yukiko and others, 2003
	Short-neck clam	100 (30/30)	
	Horse mackerel	85.8 (6/7)	
Vietnam*	Sand crab	32.5(41/126)	Wong and others, 1999
Hong Kong*	Lobster	44.1(26/59)	
Thailand*	Crawfish	21.1(20/95)	
Indonesia*	Snail	44.3(47/106)	
	Crab	71.1(81/114)	
	Crab	81.3(26/32)	
	Shrimp	75.8(47/62)	
	Fish	29.3(27/92)	

*original countries of the imported seafood in Taiwan.

objective of this literature study is to provide a scientific overview of intervention methods for reducing VP in seafood, in order to evaluate the effectiveness of potential inactivation strategies that could be used during seafood processing and consumption.

Intervention Methods

There are various methods to control bacteria in seafood. Depending on the processing needs and the consumer preference, each method has distinct advantages and limitations. For example, as a traditional method, thermal processing can inactivate microorganisms effectively, but can also induce the adverse effects on the nutrition and sensory characteristic of foods (Awuah and others 2007). Most chemical treatments, such as hypochlorite, ozone, and chlorine dioxide (ClO_2), have high antimicrobial activity and low cost, but might cause residues posing threat to human health (Olmez and Kretzschmar 2009). Nowadays, it is critical to develop effective intervention methods to eliminate VP in seafood and retain the fresh color and flavor of the products.

Physical methods

Relaying and depuration. Relaying and depuration are traditional methods for reducing bacteria and sand from seafood. Relaying is a process before harvest, in which the seafood is transferred to a clean environment from polluted areas for natural biological purification. Son and Fleet (1980) investigated the elimination of bacteria from oysters by relaying in a nonpolluted waterway and found VP could be cleansed from an initial artificial contamination of 4 log CFU/g to undetectable level (<5 cells/g) after 6 d. However, the application of relaying is limited due to the lack of clean marine environment (Su and Liu 2007).

Depuration has been recognized as a postharvest treatment to reduce pathogens and increase the shelf life of seafood products for a long history (Su and Liu 2007; Chae and others 2009). It is a controlled process that can be held in a closed system with recycled seawater to allow seafood purge bacteria in clean seawater. However, it was reported this process was limited in reducing *Vibrios* due to bacteria colonization in the intestinal tracts of seafood (Vasconcelos and Lee 1972). To achieve better decontamination effect, it is necessary to use it in conjunction with other inactivation treatments, such as refrigeration, ultraviolet light, and disinfectants. Chae and others (2009) reported that depuration in artificial seawater at 15 °C was more efficient than that at 22 °C in reducing VP from American oysters with the initial inoculation level of 5.78 log MPN/g, and a reduction of 2.1 and 3 log MPN/g was achieved after 48 and 96 h of depuration at 15 °C, respectively. Different results could be obtained depending on the initial inoculated bacterial level, bacterial strains, sanity of the depuration water, and some other factors. For example, Tamplin and Capers (1992) reported that populations of *V. vulnificus* in oyster tissue after 5 d of depuration at 15 °C remained at a level similar to pretreatment counts (4 log MPN/g); Phuvasate and others (2012) reported that VP was reduced by 2.43 log MPN/g when depurating the laboratory-contaminated oysters with the initial contamination level of 6.3 log MPN/g in artificial seawater for 5 d at 15 °C. The depuration efficacy could be improved when low temperature was used in combination with ultraviolet irradiation or disinfectants. Su and others (2010) observed 3.0 log MPN/g reduction of VP in Pacific oyster when they were depurated with refrigerated seawater combined with 15-w gamma UV sterilization for 96 and 144 h in winter and summer, respectively. Wang and others (2010) reported a reduction of 3.1 log CFU/g of VP in artificially inoculated oysters (initial contamination level of 6.2 log CFU/g) could be achieved by depuration with 20 mg/L of ClO_2 for 30 min; and after 6 h of depuration, the shelf life of oysters was extended to at least 12 d at 4 °C.

Temperature control. It has been well documented that temperature is one of the most important extrinsic factors influencing the growth and survival of VP (Drake and others 2007). The occurrence of VP is positively related to the water temperature (Shen and others 2009). As shown in Table 2, temperature control, either through thermal processing or cold storage, could effectively reduce the contamination level of VP in seafood.

Mild heat treatments are preferred considering that high temperature may greatly affect the sensory characteristics of seafood (Andrews and others 2003b; Su and Liu 2007). Andrews and others (2000) developed the low-temperature pasteurization by exposing the shell stock oysters in 55 °C water for 5 min that achieving an 48 to 50 °C internal temperature of oysters and reduced VP by 10^5 CFU/g to nondetectable level (<3 MPN/g). To reduce the highly heat-resistant *Vibrio* strain, VP O3:K6, a total processing

Table 2–Temperature control to reduce *V. parahaemolyticus* in seafoods.

Method	Seafood	Temp. (°C)	Time	Reduction	Reference
Low-temperature pasteurization	Oyster	50	10 min	5 log MPN/g	Andrews and others, 2000
		52	22 min	4 to 6 log MPN/g	Andrews and others, 2003b
Heating	Granulated ark shell clam	50	10 min	2.27 MPN/g	Liu and others, 2010
		60	5 min	>4 log MPN/g	
		70	2 min	>4 log MPN/g	
		80	1 min	>4 log MPN/g	
Frozen		−18	15 d	4.05 MPN/g	
		−30	30 d	4.05 MPN/g	
Refrigeration	Oyster	3	14 d	0.8 log CFU/g	Gooch and others, 2002
Refrigeration	Crab meat	5	14 d	5 log CFU/g	Ray and others, 1978
Refrigeration	Fish fillet	4	9 d	2 log CFU/fillet	Vasudevan and others, 2002
		8		1 log CFU/fillet	
Frozen		−18	49 d	3 log CFU/fillet	
Refrigeration	Oyster	5	96 h	1.42 log MPN/g	Shen and others, 2009
	Shucked oyster			2.0 log MPN/g	
Frozen	Oyster	−30	75 d	3.8 log MPN/g	
	Shucked oyster			5.08 log MPN/g	
	Shucked oyster	−18	60 d	5.46 log MPN/g	

time of at least 22 min at 52 °C (a 50 °C internal temperature was achieved after 13 min) was recommended to reduce the bacteria in oysters from more than 5 log MPN/g to less than 3 MPN/g (Andrews and others 2003a). However, Liu and others (2010) found that heating the granulated ark shell clams at the water temperature of 50 °C for 10 min only reduced VP by 2.27 log MPN/g from the initial level of 5 log MPN/g and concluded that low-temperature pasteurization was not very effective in reducing VP in granulated ark shell clams. However, the authors did not evaluate the internal temperatures of clams, the heating profiles, and the temperature distribution of the heating equipment, which could be important factors influencing the intervention effect of the thermal process. Mild heating used in combination with other methods could improve the efficacy of bacterial inactivation. Wang and others (2013b) obtained greater reductions of VP in shrimps when the thermal treatment was used in combination with ultrasound. Bacterial reductions of 1.76, 2.63, and 4.01 log CFU/g were obtained when the shrimps were treated with ultrasonic powers of 96, 150, and 204 W at 47 °C for 8 min, respectively, which were greater than the 0.80 log CFU/g reduction achieved by the thermal treatment alone.

Kaneko and Colwell (1973) found the minimum growth temperature of VP was 10 °C below which the bacterial population declined gradually. As shown in Table 2, refrigeration storages of seafood, such as oysters, crabs, and fish could lead to a moderate decline of VP, and the inactivation effects was found variable in different seafood species and bacterial strains. In general, refrigeration is an effective method to inhibit bacterial growth in seafood during home storage, while frozen storage has been widely adopted in the commercial delivery of seafood products since bacteria would experience a rapid decline in a short time. It is worth noting that the U. S. Food and Drug Administration (FDA) and the Interstate Shellfish Sanitation Conference (ISSC) proposed that the initial level of *Vibrios* should be between 10000 and 100000 to assure the postharvest process is capable of reducing *Vibrios* by 3.52 logs resulting in a final concentration of <30 in shellfish (FDA/NSSP, 2011). Shen and others (2009) showed the populations of VP in shell oyster were decreased from 5.46 log MPN/g to nondetectable (<3 MPN/g) after storage at −18 °C for 60 d, while 75 d were needed at −30 °C to achieve the same level of reduction. Similarly, Liu and others (2010) observed that the same reduction level of 4.05 log MPN/g of VP in the granulated ark shell clams could be achieved when frozen at −18°C for 15 d, or at −30 °C for 30 d. The greater bacterial inactivation effect at a higher freezing temperature (−18 °C) than at a lower one (−30 °C) could be probably due to the larger intracellular ice crystals formed in bacterial cells at higher freezing temperatures, causing the disruption of cell membrane, cell wall, and internal structure that led to the bacterial cell damage (Jay and others 2005). Studies indicated that pathogen might be sublethally injured during cold storage and can recover themselves and proliferate when temperature increased, and for that reason the seafood was suggested to be quickly cooled after harvest and adequately cooked before consumption to reduce health risk (Vasudevan and others 2002; Shen and others 2009).

Irradiation. Sources of radiation (including radioactive isotopes, particle accelerators, and X-ray machines) intended for use in processing food was defined as "food additives" in the legislation of Food Additives Amendment in 1958 (Morehouse 2002). Food irradiation was recognized as a physical nonthermal intervention technology initially used to inactivate food spoilage organisms for food preservation (Bolder 1997; Dinçer and Baysal 2004). Food irradiation has increasingly become an effective measure to eliminate pathogen in food using ionizing radiations including gamma rays, electron beam, and X-rays in order to prevent the incidence of foodborne diseases (Diehl 2002). The inactivation effect of irradiation on microbes was probably due to the direct damage of DNA of living organisms, inducing cross-linkages and other changes that make the organism unable to grow or reproduce (Tauxe 2001). Besides, Abdallah and others (2010) suggested that gamma irradiation could alter several outer membrane proteins (OMPs) of VP. As shown in Table 3, VP is vulnerable to a relative low irradiation dose (<3 kGy) at which a considerable bacterial reduction could be achieved in seafood.

The safety of irradiated food products is concerned and has been investigated by many international organizations and regulatory agencies, including World Health Organization (WHO), FAO, and the Codex Alimentations Commission (CAC). The application of irradiation in food has been approved by many countries around the world and their limit of use has been regulated (Steele 2000; Morehouse 2002). The maximum absorbed irradiation dose used in fish and shell fish in the United Kingdom and Belgium is 3 kGy; frozen, peeled, or decapitated shrimps could be treated no more than 5 kGy in France and Belgium; and in Holland, the irradiation dose used in shrimps should not exceed 3

Table 3–Effects of irradiation on reduction of *V. parahaemolyticus* in different seafoods.

Method	Seafood	Dose (kGy)	Bacterial reduction	Reference
X-ray	Whole shell oyster	3.0	4.3 log MPN/g	Mahmoud and Burrage, 2009
X-ray	RTE shrimp	3.0	7.6 log CFU/g	Mahmoud, 2009
Gamma irradiation	Frozen shrimp	0.1 to 0.3	D_{10}	Bandekar and others, 1987
Gamma irradiation	Salted, seasoned, and fermented oyster	0.29	D_{10}	Song and others, 2009a
Electron beam irradiation		0.29		
Gamma irradiation	Salted, seasoned, and fermented short-necked clam	0.29	D_{10}	Song and others, 2009b
Electron beam irradiation		0.36		
Gamma irradiation	Oyster	1.0	6 log CFU/g	Jakabi and others, 2003

kGy (Arvanitoyannis and others 2009). In the United States, Food and Drug Administration (FDA) has approved the use of ionizing radiation for the control of VP in fresh or frozen molluscan shellfish with an absorbed dose less than 5.5 kGy (FDA 2011a).

The application of irradiation has gained popularity because of the benefits that the food products could be processed in frozen to avoid thawing, no residue left in the food, and foods can be treated in different states, such as liquid, solid, and semisolid (Farkas 1998). Another advantage of irradiation is that little change of the sensory properties at low irradiation dose was found in seafood. Song and others (2009a) reported that there was no significant difference on sensory characteristics of oysters, not only immediately after irradiation with 0 to 5 kGy, but also during the storage at 10 °C for 4 wk. Jakabi and others (2003) found that an irradiation dose of 1.0 kGy could effectively inactivate VP in oysters without adversely affecting the sensory properties. Andrews and others (2003b) showed that VP TX-2103 (serotype O3:K6) required 1.0 to 1.5 kGy for reduction to nondetectable levels from the initial inoculated level of 6 log CFU/g, and 146 volunteers were unable to distinguish nonirradiated from irradiated oysters in the sensory test ($P < 0.001$).

High pressure. High pressure (100 to 900 MPa) is a nonthermal process that can be used to destroy pathogen in seafood and therefor prolong the shelf life of seafood products (Martin and others 2002; Murchie and others 2005). Several studies indicated that the inactivation mechanisms of high pressure process (HHP) was possibly due to the disruption of cell membrane, the changes in morphology and the internal organizations of cells, and the degradation of bacterial DNA (Chilton and others 1997; Murchie and others 2005). In addition, it was suggested that Gram-negative bacteria be more susceptibility to high pressure for the complexity of cell membranes (Shigehisa and others 1991). Chen and others (2006) reported that *Vibrio spp.* could be inactivated by treatments lower than 350 MPa and were relatively sensitive to pressure compared with other pathogens such as *Listeria spp*. The inactivation effect of HHP against VP in seafood products is summarized in Table 4. In general, high pressure treatments could achieve considerable bacterial reduction in seafood. The bacterial inactivation effect of high pressure depended on the pressure level, temperature, treatment time, and physiological state of microorganisms, among which temperature is a significant and controllable factor during the process. Kural and others (2008) showed that elevated temperature above 30 °C could enhance the bacterial inactivation, which was in agreement with the reports on *L. monocytogenes*, *Salmonella enterica*, and other microorganisms. After the high pressure processing, a considerable shelf life of seafood could be achieved. Ma and Su (2011) found that an HHP of 293 MPa for 120 s at groundwater temperature (8 ± 1°C) caused a reduction of VP in oysters by more than 3.52 log MPN/g, and achieved a shelf life of 6 to 8 and 16 to 18 d when stored at 5 °C and in ice, respectively. Romero and others (2004) reported that oysters processed with 400 MPa at 20 °C for 5 min had a shelf life of 21 d when stored in ice.

The changes in sensory characteristics of seafood treated with high pressure have been studied for many years. In general, cooked appearance, higher pH values, and protein denaturation were observed in HP-treated raw seafood, especially with higher pressures. Murchie and others (2005) reviewed the changes of fresh shellfish following high pressure treatment, and concluded that the appearance changes were largely dependent on the pressure level and the muscle became whiter and more opaque with increased pressure. Romero and others (2004) investigated the effects of high pressure treatment (at 100 to 800 MPa for 10 min at 20 °C) on the physicochemical characteristics of fresh oysters and found that the changes in color were primarily observed at pressures above 300 MPa due to the protein denaturation and the extent of color change increased gradually with the increase of treatment pressure. Besides, the changes of seafood sensory characteristics were highly dependent on the types of seafood due to the differences in denaturation resistance of proteins. Mckenna and others (2003) did not observe significant color changes in cooked salmon treated by a high pressure of 414 MPa for 5 min at 21 °C, in contrast to the significant color changes in fresh salmon treated by a high pressure of 300 MPa for 15 min at room temperature reported by Yagiz and others (2009). As reported by Matser and others (2000), high pressure treatments higher than 150 to 200 MPa for 5 min resulted in a cooked appearance of pollack, mackerel, tuna, cod, salmon trout, carp, plaice, and anglerfish, while only octopus retained a raw appearance until 400 to 800 MPa. Lakshmanan and others (2007) applied high pressure to fresh and cold smoked salmon and indicated that the increase of moisture content in cold smoked salmon was higher than that in the fresh salmon samples. In addition, the high pressure treatment reduced the water-holding capacity of fresh salmon by 5% at 200 MPa for 10 min, whereas the treatment did not cause much change in the water-holding capacity of cold smoked salmon.

Chemical methods

Electrolyzed oxidizing (EO) water. EO water was originally developed in Japan for medical utilization and has been introduced as a new antimicrobial agent used in food (Shimizu and Hurusawa 1992). Liao and others (2007) reported that high oxidation–reduction potential (ORP) could damage the outer and inner membranes of a cell and lead to the necrosis of *E. coli*. Koseki and others (2001) and Len and others (2000) suggested that the available chlorine, mainly HOCl, might be the primary factor responsible for the bactericidal potency.

EO water could be generated through electrolysis of a dilute salt solution (0.05 to 0.2% NaCl) in an electrolytic ambient. Two types of EO water can be produced, one is strong acid electrolyzed

Table 4–Effect of high pressure processing to reduce *V. parahaemolyticus* in different seafood products.

Medium	Pressure (MPa)	Time (min)	Temp. (°C)	Reduction	Reference
Clam juice	172	10	23	6.0 log CFU/mL	Styles and others, 1991
Oyster	200	10	25	6.0 log CFU/g	Berlin and others, 1999
Pure culture	345	1	21	7.4 log CFU/g	Calik and others, 2002
Oyster		2		6.2 log CFU/g	
Oyster	300	3	28	5.0 log CFU/g	Cook, 2003
Oyster	345	7.7*	21	4.5 log CFU/g	Koo and others, 2006
Oyster	350	2	1	5.4 log MPN/g	Kural and others, 2008
			20	5.3 log MPN/g	
			35	6.5 log MPN/g	
Oyster	293	2	8	3.52 log CFU/g	Ma and Su, 2011

*includes a 6.7-min pressure come-up time.

Table 5–Effect of EO water on *V. parahaemolyticus* in different seafood products.

	Solution properties						
Seafood	pH	OPR (mV)	Available chlorine (mg/L)	Temp	Exposing time	Reduction	Reference
Cooked shrimp	2.43	1135.9	36	4 °C	1 min	0.5 log CFU/g	Xie, 2011
					5 min	0.68 log CFU/g	
	2.40	1133.8	43	20 °C	1 min	0.45 log CFU/g	
					5 min	1.0 log CFU/g	
	2.38	1127.1	21	50°C	1 min	2.12 log CFU/g	
					5 min	3.11 log CFU/g	
Raw oyster	2.82	1131	30	room temp	2 h	0.87 log MPN/g	Ren and Su, 2006
					4 h	1.13 log MPN/g	
Tilapia	2.47	1156	120	room temp	5 min	1.49 log CFU/g	Huang and others, 2006
					10 min	2.61 log CFU/g	

water (StAEW) produced in an ambient with a diaphragm to separate the anodic and cathodic chambers, and the other is weakly acidic electrolyzed water (WAEW), or slightly acidic electrolyzed water (SAEW) produced in an ambient without a diaphragm (Quan and others 2010). StAEW was characterized by low pH (2.2 to 2.7), high OPR (>1000 mV), and high available chlorine concentration (ACC, up to 120 ppm). It has been validated to possess strong bactericidal activities against a variety of foodborne pathogens (Huang and others 2008). As shown in Table 5, StAEW was presented as a potential decontaminants for reducing VP in seafood, especially using AEW with higher concentrations of available chlorine or combined with higher temperatures. In recent years, WAEW is becoming more preferred for application in the food industry because its mild pH (5 to 6.5) could reduce the corrosions of the platform surfaces and public health risks. Quan and others (2010) reported that VP were reduced from the initial concentration of 5.7 log CFU/mL to nondetected levels in cell suspensions treated with WAEW (pH: 5.9; ORP: 798 Mv; ACC: 35 ppm) for 30 s. However, the bactericidal effect of WAEW on VP in seafood products has rarely been reported.

The main advantages of EO water are environmental friendly, more economical, and no adverse impact on human health compared to other chemical disinfectants (Sakurai and others 2003), while the solution would rapidly loses its antimicrobial activity if it is not continuously supplied with H^+, HOCl, and Cl_2 by electrolysis (Kiura and others 2002), which is a great challenge for its application in the food industry.

Chlorine and ClO_2. Chlorine was first used as a disinfectant for the treatment of polluted water in 1897 and then introduced as a decontamination agent in seafood industry in 1935 (Fitzgerald and Conway 1937). More than 90% of VP in artificially contaminated shrimps (inoculated with 8 log CFU/mL bacterial suspension) could be reduced when they were treated with chlorine at the concentration of 50 ppm and for contact time of 30 min (Chaiyakosa and others 2007). In recent years, the concerns of the potential health hazard of chlorine to human have arisen. First, a long time of contact would lead to the severe respiratory tract damage to industry workers. Second, the by-products, trihalmomethanes (THMs), which would be produced when chlorine reacted with the organic compounds in food, appeared to be mutagenic (Owusu and others 1990; Andrews and others 2002). Therefore, the application of chlorine in seafood processing carried a potential health risk to consumers.

ClO_2 is a strong oxidizing agent with the antimicrobial capacity against a variety of foodborne pathogens and has been widely used as an alternative disinfectant to chlorine. Studies have shown the satisfactory microbial decontamination effect of ClO_2 in drinking water (Shams and others 2011) and seafood (Kim and others 1999; Andrews and others 2002). The U.S. Environmental Protection Agency (EPA) has conducted a two-generation reproduction study and derived an acceptable daily intake of 0.03 mg/kg/day for ClO_2 and chlorite (EPA 2000). Application of ClO_2 as a decontamination agent has been approved for cleaning potable water and fresh-produce products in the European Union and the United States, respectively (EU 2004; FDA 2008). Several researchers studied the antimicrobial effect of ClO_2 in the seafood depuration process. Puente and others (1992) found that ClO_2 could be a candidate disinfectant in seafood depuration for controlling *VP* in seawater and suggested it should be evaluated as a potential disinfectant for aquaculture. Wang and others (2010) used ClO_2 as a disinfectant in oyster depuration for removing VP and reported that the bacterial could be eliminated completely after 6 h of treatment with 20 mg/L of ClO_2. Ramos and others (2012) investigated the decontamination effect of UV light used in combination with chlorinated seawater for removing VP and the results showed a bacterial reduction of 3.1 log MPN/g after 48 h of depuration.

Organic acids. Some of the commonly used organic acids, such as lactic acid, benzoic acid, and acetic acid have been generally recognized as safe (GRAS) (FDA 2011b) and traditionally utilized as food additives and preservatives to extend the shelf life of perishable food (Gould 1996; Ricke 2003). Although the antimicrobial activities of organic acid have been investigated, the antibacterial mechanisms are not fully understood due to the complex metabolism process occurred in bacterial cells. The organic acids are assumed capable of penetrating the lipid membrane and dissociating into anions and protons in the cell in which a neutral pH cytoplasm must maintained to sustain metabolism function (Davidson 2001). Once internalized into bacterial cells, the organic acids could increase the cellular osmolarity, inhibit the biomacromolecules synthesis and induce the antimicrobial peptide in host cells (Hsiao and Siebert 1999; Ricke 2003; Brogden 2005).

Among these various organic acids, lactic acid has been primarily reported as an effective sanitizer used in seafood. Shirazinejad and others (2010) evaluated the intervention effect of lactic acid against VP in fresh shrimps, showing that the populations of VP inoculated into shrimps were reduced by greater than 2 log CFU/g after dipping in 3% (v/v) lactic acid for 10 min and no adverse change of shrimp sensory properties was observed. The survival of VP in artificially contaminated mussel dipped in 1% (v/v) lactic acid for 15 min was investigated and a bacterial reduction of greater than 3.38 log CFU/g could be achieved (Terzi and Gucukoglu 2010). Less bacterial reduction in seafood was observed using acid solutions with higher pH. Mejlholm and others (2012) marinated the shrimps in the brine (pH 4.0) containing 0.21% (w/v) of benzoic acid, 1.65% (w/v) of citric acid, and 0.1%% (w/v) of sorbic acid and observed a reduction of 0.9 log CFU/g of VP after 24 h, while no reduction was found in shrimps marinated in the brine (pH 4.9) containing 1.39% (w/v) of acetic acid and1.86% (w/v) of lactic acid.

Chitosan. Chitosan, mainly composed of 2-amino-2-deoxy-D-glucose, is a group of biopolymers derived by deacetylation of chitin which is rich in the shells of crustaceans (Devlieghere and others 2004). Chitosan was initially used as a food preservative by coating the food surface against spoilage microorganisms and during the past several decades, it has been recognized as a natural disinfectant against a wide range of bacteria, fungi, and yeasts (Rabea and others 2003; Devlieghere and others 2004; No and others 2007). Several hypotheses have been proposed to elucidate the bactericidal mechanism of chitosan. The predominant assumption is that the reaction of positive charged chitosan molecules and negative charged cell membranes causes the leakage of functional components, consequently leading to the cell destruction (Sudarshan and others 1992; Kong and others 2010; Wang and others 2013a). Another explanation was attributed the detrimental effect to the binding between chitosan and the DNA in microbial cells, causing the inhibition of mRNA and protein synthesis (Sudarshan and others 1992; Rabea and others 2003). The antimicrobial effect of chitosan depends on its molecular weight, degree of deacetylation, bacterial strains, and food matrix (Devlieghere and others 2004; Kong and others 2010; Alishanhi and Aïder 2012).

Chaiyakosa and others (2007) reported more than 60% reduction of VP in raw shrimps from an initial inoculation level of 8 log CFU/mL when they were treated with chitosan (85% degree of deacetylation and molecular weight of 161 kDa) at the concentration of 1000 ppm for 120 min. Terzi and Gucukoglu (2010) investigated the decontamination effect of chitosan against VP in mussel samples, showing that dipping treatment in 0.05%, 0.1%, 0.25%, and 0.5% of chitosan (molecular weight = 150 kDa, 75% to 85% deacetylation, viscosity 20 to 200 cps) for 5 min could reduce the bacteria by 1.33, 1.41, 1.56, and >2.03 log CFU/g, respectively. Alishanhi and Aïder (2012) reviewed the applications of chitosan in seafood processing and suggested that it could be successfully incorporated into seafood products to improve food safety. The mainly advantage of chitosan is that it could be used as potential disinfectant with no adverse health effects to human. In Korea and Japan, chitosan has been approved as a food additive since 1995 and 1983, respectively (Weiner 1992; KFDA 1995). Shrimp-derived chitosan has been approved for use in food as a processing aid, nutrient supplement, stabilizer, thickeners, emulsifier, and antimicrobial agents in accordance with good manufacturing practice in the United States (FDA 2012).

Essential oils. Essential oils, also named volatile or ethereal oils, are natural extracts obtained by distilling from plants, such as spices, herbs, garlic, flowers, and buds. Compared with artificial chemicals or synthetic additives, essential oils have been recognized as effective decontaminants against various pathogens without adverse effects to human health (Burt 2004). The antimicrobial activity of essential oils is primarily attributed to the phenolic components comprising more than 60 individual components (Beuchat 1993; Russo and others 1998; Cosentino and others 1999). Due to the complicated constituent of essential oils, the inactivation mechanism has not been explained clearly. Burt (2004) indicated that the antibacterial action mode was probably attributed to the hydrophobicity of essential oils, which could disrupt the lipids of the cell membrane and mitochondria, disturbing the structure and rendering them more permeable.

Essential oils from various plants exhibited potential antimicrobial effects against VP. Vuddhakul and others (2007) evaluated the antibacterial activities of 13 Thai condiments against VP using the disk diffusion method, showing that the fresh squeezed extracts from galangal, garlic, and lemon at a concentration of 10 mL/disk produced a clear bacterial inhibition zone of 13.6 ± 0.5, 11.6 ± 0.5 and 8.6 ± 1.2 mm, respectively. Yano and others (2006) added basil, clove, garlic, horseradish, marjoram, oregano, rosemary, thyme, and turmeric into sterile natural seawater at 30 °C to inhibit the growth of VP in seafood. Their results showed that the minimum inhibitory concentrations of these essential oils were 0.016%, 0.004%, 0.25%, 1%, 0.001%, 0.032%, 0.008%, 0.032%, and 2%, respectively, suggesting that all of them could be used to protect seafood from VP contamination. Although a number of studies investigated the potential inactivation effect of essential oils against VP, the application in seafood is very limited to our knowledge. Lin and others (2005) treated the fish slices with a mixture (1:1) of oregano and cranberry extract containing 0.1 mg/mL phenolic, and found that after storage for 8 d at 4 °C, VP in fish slices was reduced by more than 3 log CFU/g.

Biological methods

Probiotics. Probiotics, a group of live microorganisms that confer a health benefit on the host when they are consumed in adequate amounts as part of food (FAO/WHO 2001), have been widely used as feed additives in aquaculture to support the health of aquatic animals (Wang and others 2008). Probiotics organisms can act as antimicrobials by disrupting virulence gene expression, bacterial attachment, and cell-to-cell communication of pathogenic bacteria. The antimicrobial effects of probiotics could probably be attributed to the produced inhibitory compounds, including lytic enzymes, iron-chelating compounds, antibiotics, hydrogen peroxide, organic acids, and bacteriocins (Teplitski and others 2009). Bacteriocin-producing lactic acid bacteria were considered as the predominant probiotic microorganisms in aquaculture. Hwanhlem

Table 6–Advantages and limitations of intervention methods for reducing *V. parahaemolyticus* in seafood.

Intervention methods	Advantages	Limitations
Relaying	• Natural biological process without seafood injury	• Not so effective in limited time • Lack of clean and unpolluted marine environment (Su and Liu 2007)
Depuration	• Long history of use (Chae and others 2009) • Avoid the death of seafood	• Need to use in conjunction with other methods to achieve better efficacy
Thermal treatments	• Effective inactivation with a short-time treatment • Mild heat treatment widely used with little sensory change (Andrews and others 2003b)	• High temperature leading the protein denaturation • Costly
Refrigeration and frozen	• Effectively inhibit the growth of the bacteria during the long-time storage	• Temperature dependent
Irradiation	• No chemical residues • No health risk under the limited dose	• Costly
HHP	• Effective inactivation with a short time treatment	• Costly • Negative sensory affect (Murchie and others 2005)
EO water	• Environmental friendly (Sakurai and others 2003) • Effective antimicrobial activity at neutral pH • Low cost	• Stability • Relatively low inactivation efficacy in a short time • Affected by the temperature
Chlorine and chlorine dioxide	• Effective antimicrobial activity • For chlorine dioxide: less pH dependent than chlorine; less corrosive • Low cost	• Not permitted for seafood processing • For chlorine: potential adverse health effects; corrosive to equipment; pH dependent
Organic acids	• Easy to use • No adverse health threat (FDA 2011b)	• Relatively low inactivation efficacy • Long contact time • pH dependent
Chitosan	• No adverse health threat • No interferes with sensory • Use to prolong the shelf life of seafood	• Relatively low inactivation efficacy
Essential oils	• No adverse health threat (Burt 2004) • Advanced sensory	• Relatively low inactivation efficacy
Biocontrols	• No chemical residue	• Limited researches concerning the public reaction to the biocontrol used in seafood processing

and others (2010) reported that probiotic lactic acid bacteria could completely inhibit the growth of *V. parahaemolytius* within 24 h of incubation. Xi (2011) added the lactic acid bacteria (*Lactobacillus plantarum* ATCC 8014) to artificial seawater for depuration of Pacific oysters and found a significant reduction of VP (by more than 3.42 MPN/g) in oysters after 5 d of depuration at 10 ± 1 °C, indicating the lactic acid bacteria can be applied in the seafood depuration at low temperatures to reduce VP.

Bacteriophages. Bacteriophages are viruses that enable their nucleic acids to invade bacterial cells, self-replicate and cause the lysis of cells. Since the application of a bacteriophage-based additive for the control of *L. monocytogenes* in food was approved by the U.S. FDA in 2006, the applications of bacteriophage as a biocontrol agent have been increasing (Mahony and others 2011). García and others (2008) reviewed the application of bacteriophages in food and found they were effective to control the contamination of *E. coli* O157: H7, *Salmonella*, *Campylobacter*, *L. monocytogenes*, and *Staphylococcus aureus*. Phages are extremely host-specific, and the phages specific for VP are abundant in marine environment (Jiang and Paul 1994). Silva (2005) evaluated the effectiveness of *V.* phages in reducing VP in oysters and showed that the reduction of *VP* was nearly 2 log CFU/g in 5 to 30 min. Although currently bacteriophages are generally studied as antimicrobial agents at the experiment stage, bacteriophage-based methods are promising to be an alternative intervention technology in the seafood industry.

Other methods investigated for nonseafood

Apart from the above-mentioned intervention methods, there are a number of novel technologies that have shown effectiveness to inactivate VP in seawater and other food products, although not directly in seafood. These technologies may be adopted to inactivate VP in seafood in the future after more researches are conducted. For instance, the electric current treatment has been used to inactivate VP in seawater. Park and others (2003) found that low-amperage electric treatment (100 ms by a 0.5 to 2 A, 12-V direct current) could completely eliminate VP in seawater with the contamination level of 1.0×10^3 cells/mL. Urano and others (2006) verified that the superior inactivation efficacy against VP in saline solution by direct-current electric treatment, and drew a conclusion that the generation and accumulation of oxidized halogen compounds was the essential reason for the inactivation of VP during the treatment. Park and others (2004) investigated the alternating current (AC) treatment against VP in seawater with the initial contamination level of >1000 CFU/mL, showing that voltage of 3 A at frequencies of 5, 16, and 50 Hz could completely inactivate the bacteria. Furthermore, the authors suggested that AC treatment could reduce the generation of chlorine gas and would be more environmental friendly and suitable for practical industrial application.

Chemical compounds derived from plant, animal, or microbial origins have also been studied for their antimicrobial effects against VP. Sicairos and others (2009) showed that the halophilic pathogenic (O3:K6 strain) and a multidrug resistant isolate strains (strain 272) of VP were sensitive to the lactoferrin chimera and more than 95% growth was inhibited when the cell cultures (an optical density 660 nm of 0.005) were treated with 40 μM lactoferrin chimera. Genovese and others (2012) observed a moderate antimicrobial activity of an ethanol extract from *Asparagopsis*

taxiformis against VP with a 12.0 ± 3.5 mm inhibition zone, suggesting that *A. taxiformis* extracts could be an alternative antimicrobial agent used both in the storage of mollusks and discharged seawater.

Other alternative technologies, such as pulse light, oscillating magnetic fields, and bromine, have been successful to eliminate food pathogens in various foods (Parish and others 2003). Although no reports on these technologies used for seafood, further research may prove their use alone or in combination with other methods discussed above to effectively inactivate VP in seafood products.

Conclusions

V. parahaeomolyticus is widely distributed in the marine environment and frequently associated with the outbreak of illness in seafood, posing a serious risk to the public health. This paper reviewed the antimicrobial effectiveness of various intervention methods to control, either reduce or eliminate, this bacterium in seafood products. Advantages and disadvantages of each method are summarized in Table 6. Traditional intervention methods such as thermal treatment and high pressure processing, could be effective to inactivate VP in seafood, but may cause undesirable flavors and odors that could not meet consumers' increasing demand for minimally processed food. Implementation of natural antimicrobials as preservatives for seafood will dramatically increase in the future due to consumers' increasing preference for raw and lightly cooked seafood, as well as the more stringent restrictions on the use of synthetic antimicrobials in food products. However, since the bacterial inactivation effect of natural antimicrobials is limited, research on the combined use of natural antimicrobials with other hurdle factors should be conducted in the future.

In order to minimize the risk of VP for human health and simultaneously keep the flavor and nutritious aspects of seafood, future research on the intervention strategies against VP may endeavor in the following areas:

- More researches on the nonthermal physical strategies, which are characterized as low cost, easy to use and little interferes with sensory properties, are needed to inactivate VP during the seafood washing process, such as pulse light and oscillating magnetic fields.
- The antimicrobial mechanisms of many chemical agents are not fully understood. Research on the antimicrobial mechanisms would be helpful for the development of effective antimicrobial treatments to reduce VP in seafood.
- Considering the potential synergistic bactericidal effect, further studies are needed to investigate novel combinations of disinfectants and/or physical intervention methods to control VP in seafood processing.
- As VP is ubiquitous in the marine environment, it is important to take effective measures to prevent the potential proliferation of the bacteria along the seafood production and processing chain.
- Since the recovery of the bacterial cells sublethally injured during the inactivation treatment poses a potential food safety risk, strategies to prevent the bacterial recovery would enhance the antimicrobial efficacy of intervention methods.
- Quantitative microbial predictive modeling and risk assessment should be used to evaluate the effectiveness of intervention strategies on reducing or eliminating VP in seafood.

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