



A predictive model for assessment of decontamination effects of lactic acid and chitosan used in combination on *Vibrio parahaemoliticus* in shrimps



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ARTICLE INFO

Article history:

Received 26 September 2012

Received in revised form 14 May 2013

Accepted 14 July 2013

Available online 19 July 2013

Keywords:

Response surface model

Vibrio parahaemoliticus

Shrimp decontamination

Lactic acid

Chitosan

ABSTRACT

Vibrio parahaemoliticus is a major causative agent of human gastroenteritis in seafood products including shrimps. Lactic acid and chitosan are natural antimicrobials for food decontamination in the washing process of seafood. In this research, a 4-factor response surface model based on the Box–Behnken experimental design was developed to evaluate the effects of lactic acid (1%, 2%, and 3%, v/v), chitosan (0.4%, 1%, and 1.6%, w/v), rotational rate (90, 110, and 130 rpm) and washing time (10, 20, and 30 min) on reduction of *V. parahaemoliticus* inoculated in raw shrimps. These treatments achieved 2.2 to 4.3 log₁₀ CFU/g reduction of *V. parahaemoliticus* in shrimps. Stepwise stratification led to a simplified model that has a satisfactory performance as evidenced by statistical indices ($R^2 = 0.92$; $p < 0.0001$; RMSE = 0.196) and external validation parameters [bias factor (B_f) = 1.01; accuracy factor (A_f) = 1.05]. The model generated an optimum treatment combination (3% lactic acid, 1.6% chitosan, and rotational rate at 110 rpm) that could achieve greatest bacterial reduction of 4.5 log₁₀ CFU/g. Among the four factors, lactic acid and chitosan were the major contributors for bacterial decontamination. Analysis of variances showed a significant interactive inactivation effect ($p < 0.05$) from combined use of lactic acid and chitosan. The treatments did not have adverse effects on the quality attributes such as color and pH of the shrimps.

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1. Introduction

Vibrio parahaemoliticus, a halophilic Gram-negative rod-shaped bacterium widely distributed in marine environments, has been recognized as one of the leading causes of foodborne outbreaks associated with seafood consumption around the world (Baumann and Schubert, 1984; Iwahori and Yamamoto, 2010). In recent years, consumer demand for shrimp has steadily increased throughout the world (Norhana et al., 2010). High prevalence of *V. parahaemoliticus* in shrimps has been reported in many countries, especially in Asia, including Thailand, Iran and China (Minami et al., 2010; Zarei et al., 2012; Zhang et al., 2007). A number of human gastroenteritis outbreaks have been reported associated with the consumption of raw or undercooked shrimp contaminated with *V. parahaemoliticus*, posing potential risk to public health and indicating the need to mitigate bacterial load in shrimps (Cabanillas-Beltrán et al., 2006; CSPI, 2007; Yang et al., 2008).

A variety of intervention methods have been investigated for eliminating pathogens in shrimps. Intervention methods, such as thermal treatment, high pressure and irradiation, have been reported to be alternatives to inactivate *V. parahaemoliticus* in seafood (Andrews et al.,

2000; Ma and Su, 2011; Mahmoud, 2009). However, treatments with high temperature (>52.5 °C) or pressure (>800 MPa) could lead to undesirable changes in color, flavor and/or texture (Andrews et al., 2003; Romero et al., 2004). In order to minimize the adverse effects on nutrition and sensory characteristics of food, there is a strong need to develop effective and safe intervention methods in post-harvest shrimp processing (Norhana et al., 2010).

Lactic acid has been generally recognized as safe (GRAS) under 21CFR 184.1061 by the U.S. Food and Drug Administration (FDA, 2011b) and shown as a potential disinfectant against pathogenic bacteria on beef, vegetables and fruits (El-Khateib et al., 1993; Lin et al., 2002; Venkitanarayanan et al., 2002). Treatment with 1–3% lactic acid for 10–15 min could reduce *V. parahaemoliticus* by 2–3 log₁₀ CFU/g without adverse effects on sensory properties in seafood (Shirazinejad et al., 2010; Terzi and Gucukoglu, 2010). Chitosan, rich in shells of crustaceans, such as crabs and shrimps, was initially used as a food preservative by coating the food surface against spoilage microorganisms (No et al., 2007). With regard to the antimicrobial effects of chitosan in seafood products, several studies revealed limited effect of chitosan on bacterial inactivation (Lopez-Caballero et al., 2005; Ye et al., 2008), while some other studies reported its effective antibacterial activities against a variety of pathogenic bacteria (Cao et al., 2009; Roller and Corvill, 2000; No et al., 2007; Terzi and Gucukoglu, 2010). The reason

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for the difference could be that the inactivation activity of chitosan depends on its molecular weight, degree of deacetylation, bacterial strains, and food matrix (Alishanhi and Aider, 2012; Devlieghere et al., 2004; Kong et al., 2010). Alishanhi and Aider (2012) reviewed the applications of chitosan in seafood industry and suggested that it could be successfully incorporated into seafood products to improve seafood safety.

Predictive microbiology has been used as an important tool to improve food safety by developing mathematical models to quantitatively predict the growth and survival of microorganisms under prescribed environmental conditions during food processing and storage (McMeekin et al., 1997; Whiting and Buchanan, 1994; Yang et al., 2009; Li et al., 2011). In recent years, response surface models have been used for optimization of treatment conditions for prediction of pathogen inactivation in fresh-cut vegetables, fruits and meat. The model can be used to analyze individual or combined effects of independent factors in the experiments (Bas and Boyaci, 2007; Kwak et al., 2011; Lahlali et al., 2008; Bover-Cid et al., 2012

Table 2
Experimental design and population reduction of *Vibrio parahaemoliticus* in shrimps.

Run	Variables				Reduction ^a (log ₁₀ CFU/g)
	Lactic acid (%, v/v)	Chitosan (%, w/v)	Rotational rate (rpm)	Time (min)	
1	2	1.6	90	20	3.12 ± 0.34 ^{efgh}
2	1	1	90	20	2.17 ± 0.36 ^l
3	2	1	90	10	2.61 ± 0.43 ^{ghijkl}
4	3	1	90	20	3.51 ± 0.40 ^{de}
5	2	0.4	90	20	2.44 ± 0.43 ^{hijkl}
6	2	1	90	30	2.95 ± 0.30 ^{efghijk}
7	3	1	110	30	3.98 ± 0.42 ^{abcd}
8	1	0.4	110	20	2.25 ± 0.33 ^{kl}
9	3	1.6	110	20	4.20 ± 0.68 ^{ab}
10	2	1.6	110	10	3.30 ± 0.24 ^{efg}
11	2	0.4	110	30	3.02 ± 0.37 ^{efghij}
12	3	0.4	110	20	3.12 ± 0.20 ^{efgh}
13	2	1.6	110	30	3.45 ± 0.31 ^{def}
14	1	1	110	10	2.40 ± 0.40 ^{ijkl}
15	2	1	110	20	3.11 ± 0.29 ^{efgh}
16	2	1	110	20	3.02 ± 0.20 ^{efghi}
17	1	1	110	30	2.61 ± 0.29 ^{ghijkl}
18	3	1	110	10	4.32 ± 0.41 ^a
19	2	1	110	20	3.15 ± 0.11 ^{efgh}
20	2	0.4	110	10	2.66 ± 0.51 ^{ghijkl}
21	1	1.6	110	20	2.38 ± 0.50 ^{hijkl}
22	2	1.6	130	20	3.57 ± 0.46 ^{bcd}
23	1	1	130	20	2.35 ± 0.32 ^{kl}
24	2	1	130	30	3.12 ± 0.27 ^{efgh}
25	2	1	130	10	2.75 ± 0.25 ^{efghijkl}
26	3	1	130	20	4.14 ± 0.42 ^{abc}
27	2	0.4	130	20	2.59 ± 0.41 ^{ghijkl}

^{a–l}Mean values followed by different superscript letters are significantly different ($p < 0.05$).

^a Values are means ± standard deviations ($n = 6$). The initial inoculation load of *Vibrio parahaemoliticus* in shrimps determined by positive controls was $7.2 \pm 0.3 \log_{10}$ CFU/g.

in Eq. 2 was employed to predict the reduction of *V. parahaemoliticus* (Y) in shrimps subjected to washing with lactic acid and chitosan:

$$Y = k_0 + k_1x_1 + k_2x_2 + k_3x_3 + k_4x_4 + k_5x_1^2 + k_6x_2^2 + k_7x_3^2 + k_8x_4^2 + k_9x_1x_2 + k_{10}x_1x_3 + k_{11}x_1x_4 + k_{12}x_2x_3 + k_{13}x_2x_4 + k_{14}x_3x_4 \quad (2)$$

where, Y is the bacterial reduction (\log_{10} CFU/g); x_1 , x_2 , x_3 and x_4 represent coded variables, including lactic acid, chitosan, rotational rate, and washing time, respectively. k_0 is the constant; k_1 , k_2 , k_3 and k_4 are linear coefficients; k_5 , k_6 , k_7 and k_8 are quadratic coefficients; k_9 , k_{10} , k_{11} , k_{12} , k_{13} and k_{14} are interaction coefficients. Statistical analysis of variance (ANOVA) was used to evaluate significance and adequacy of the model. Only significant variables based on the F -test results were selected by a stepwise regression to simplify the model.

To validate the model, eight additional trials with random combinations of four variables were conducted (Table 3). The method of bacterial inactivation in shrimps was the same as explained previously for the

Table 3
Eight independent trials to validate the predictive model for inactivation of *Vibrio parahaemoliticus* in shrimps.

Run	Lactic acid (%, v/v)	Chitosan (%, w/v)	Rotational rate (rpm)	Bacterial reduction (log ₁₀ CFU/g)	
				Observed	Predicted
1	2.5	1.2	100	3.05 ± 0.26	3.34
2	1.5	0.8	100	2.31 ± 0.30	2.36
3	2.5	0.8	100	3.07 ± 0.13	3.04
4	1.5	1.2	100	2.70 ± 0.19	2.50
5	1.5	1.2	120	2.62 ± 0.39	2.54
6	2.5	0.8	120	3.27 ± 0.32	3.08
7	1.5	0.8	120	2.58 ± 0.18	2.40
8	2.5	1.2	120	3.30 ± 0.46	3.38

inactivation treatments. The selected parameters were within the original range of the experimental design but not included in the establishment of the model. The observed results were used to evaluate performance of the model by the bias factor (B_f) (Eq. (3)) and the accuracy factor (A_f) (Eq. (4)) proposed by Ross (1996).

$$B_f = 10 \left[\frac{\sum_{i=1}^n \log \left(\frac{obs}{pred} \right) / n \right] \quad (3)$$

$$A_f = 10 \left[\frac{\sum_{i=1}^n \left| \log \left(\frac{obs}{pred} \right) \right| / n \right] \quad (4)$$

where n is the number of trials, obs is the observed values of bacterial reduction (\log_{10} CFU/g), and $pred$ is the predicted values of bacterial reduction (\log_{10} CFU/g).

2.7. Measurement of color and pH of the shrimp samples

Color changes of the treated shrimps were determined by placing the probe of the colorimeter Chroma Meter CR 400 (Minolta Osaka, Japan) onto the shrimp surface. Values of L , a , and b representing lightness, redness and yellowness, respectively, were recorded. The pH values of treatment solutions were measured with a Mettler Toledo pH meter (Model FE20; Toledo Instruments Co. Ltd., Switzerland), and those of shrimps were measured with a portable pH meter (Hach H160, Loveland, CO, USA) by directly inserting the probe into the shrimps. All measurements were taken on three sites of each treated sample.

3. Results

3.1. Reduction of *V. parahaemoliticus* in shrimps

The initial inoculation load in shrimps determined by positive controls was approximately $7.2 \log_{10}$ CFU/g. Microbial reduction for all combined treatments of lactic acid and chitosan, treatment time and rotational rate are summarized in Table 2. Washing with the antimicrobial solutions achieved significant reduction of *V. parahaemoliticus* in shrimps, ranging from 2.2 to $4.3 \log_{10}$ CFU/g. Among the four variables, lactic acid was the most significant to decontaminate the bacteria ($p < 0.0001$). Washing with 3% lactic acid combined with any other factors could lead to reduction by more than $3 \log_{10}$ CFU/g.

3.2. Response surface modeling of *V. parahaemoliticus* inactivation

A backward stepwise regression was carried out using JMP software to develop a simplified response surface model only with the significant variables. The lack of fit test was conducted to evaluate the systematic variations unaccounted for in the hypothesized model (a significant lack of fit indicates that the model does not describe the data well). By using ANOVA on the estimated parameters of all variables, we found statistical significance with three linear coefficients (x_1 , x_2 and x_3) ($p < 0.0001$), one interaction coefficient ($x_1 \cdot x_2$) ($p < 0.05$), and the quadratic coefficient (x_3^2) ($p < 0.05$) for estimation of bacterial reduction to the treatments. A summary of the estimated parameters for coded variables with significances is given in Table 4. The simplified model is shown as Eq. 5. The model has a good statistical performance as shown by R^2 (0.92), probability value ($p < 0.0001$) and the lack of fit test ($p > 0.05$).

$$Y = -4.66 + 0.36X_1 - 0.25X_2 + 0.11X_3 + 0.40X_1X_2 - 0.00047X_3^2 \quad (5)$$

Table 5
Changes in color and pH of the shrimps treated with lactic acid and chitosan washing.

Lactic acid (%, v/v)	Chitosan (%, w/v)	Rotational rate (rpm)	Time (min)	Color			Δ pH
				Δ L	Δ a	Δ b	
2	1	90	10	0.67 ± 1.42	1.08 ± 1.05	-1.21 ± 1.59	-0.23 ± 0.06
1	1	90	20	2.9 ± 3.35	0.48 ± 0.4	-1.81 ± 0.34	-0.27 ± 0.17
2	0.4	90	20	1.74 ± 1.85	0.49 ± 0.78	-0.89 ± 1.73	-0.08 ± 0.02
2	1.6	90	20	9.36 ± 1.88	0.98 ± 1.17	0.23 ± 1.1	-0.26 ± 0.28
3	1	90	20	3.74 ± 3.82	-1.05 ± 0.55	-3.26 ± 1.65	-0.22 ± 0.01
2	1	90	30	16.39 ± 2.42	2.94 ± 2.38	2.66 ± 2.45	-0.24 ± 0.07
1	1	110	10	5.4 ± 0.65	0.18 ± 1.03	-2.94 ± 3.13	-0.16 ± 0.06
2	0.4	110	10	15.35 ± 1.34	3.3 ± 1.83	-2.94 ± 3.13	-0.1 ± 0.13
2	1.6	110	10	12.01 ± 3.4	-0.49 ± 1.47	0.19 ± 4.4	-0.31 ± 0.25
3	1	110	10	12.59 ± 1.74	1.97 ± 1.12	-2.05 ± 1.13	0.1 ± 0.04
1	0.4	110	20	19.7 ± 0.6	2.11 ± 1.09	0.08 ± 2.31	-0.1 ± 0.08
1	1.6	110	20	4.12 ± 4.68	0.15 ± 0.23	0.96 ± 1.2	-0.08 ± 0.06
2	1	110	20	16.82 ± 2.53	2.23 ± 0.59	0.27 ± 1.21	-0.13 ± 0.04
3	0.4	110	20	18.66 ± 2.72	4.12 ± 0.86	0.61 ± 1.95	-0.16 ± 0.15
3	1.6	110	20	15.89 ± 2.73	4.36 ± 0.65	2.95 ± 2.55	-0.29 ± 0.1
1	1	110	30	12.05 ± 0.73	-0.47 ± 0.75	2.41 ± 1.86	-0.16 ± 0.07
2	0.4	110	30	22.2 ± 0.42	3.92 ± 3.39	-1.9 ± 0.95	-0.08 ± 0.05
2	1.6	110	30	17.9 ± 1.08	2.47 ± 0.76	4.63 ± 2.92	0.09 ± 0.08
3	1	110	30	21.76 ± 2.24	1.88 ± 0.69	2.14 ± 0.89	-0.2 ± 0.06
2	1	130	10	12.32 ± 3.46	0.51 ± 0.87	-1.82 ± 0.96	-0.25 ± 0.09
1	1	130	20	10.63 ± 2.51	-1.05 ± 0.07	-3.49 ± 1.08	0.03 ± 0.06
2	0.4	130	20	21.39 ± 2.55	2.61 ± 1.24	3.48 ± 1.69	-0.16 ± 0.17
2	1.6	130	20	12.08 ± 4.07	-0.34 ± 0.43	-1.6 ± 2.37	-0.2 ± 0.14
3	1	130	20	20.65 ± 1.98	2.47 ± 1.3	1.75 ± 0.9	-0.22 ± 0.14
2	1	130	30	21.54 ± 1.98	2.41 ± 1.09	1.89 ± 1.43	-0.08 ± 0.2

Values represent means ± standard deviations (n = 3).

4. Discussion

In previous studies, lactic acid has been considered as a potential decontamination agent for eliminating pathogenic bacteria in food by acting to permeabilize bacterial outer membrane and to sensitize the bacteria to detergents or lysozyme (Alakomi et al., 2000; Ricke, 2003; Jimenez-Villarreal et al., 2003; Mani-López et al., 2012). Nevertheless, treatment with high concentration of lactic acid for a long time should be avoided in seafood processing due to unacceptable sensory quality (Prakash et al., 2000). Marshall and Kim (1995) suggested that concentrations of lactic acid greater than 2% or exposure time longer than 30 s were not recommended for catfish fillets due to significant color damage. Shirazinejad et al. (2010) reported that dipping in 3% lactic acid for 10 min could reduce *V. parahaemoliticus* in shrimps by 2.5 log₁₀ CFU/g, while a significant change of sensory characteristics ($p < 0.05$) was seen when extending the dipping time to 30 min.

In our study, color changes of treated shrimps were shown as increases of lightness (Δ L) and redness (Δ a), leading to a slightly brighter and redder appearance more appealing to visual inspection. The reason suggested for color changes is the denaturation of astaxanthin, the major carotenoid pigment of shrimp (Kaur et al., 2013; Niamnuy et al., 2007). Combined use of lactic acid and higher concentration of chitosan prevented intense discoloration of shrimps by avoiding low pH. The values of Δ L and Δ a were significantly lower (indicating better sensory quality) for the combined use of 1% lactic acid and 1.6% chitosan than those of the combination of 1% lactic acid and 0.4% chitosan (pH 4.59 vs pH 2.93).

Bacterial destruction by chitosan is characterized by leakage of proteinaceous and other organelles from bacterial cells, most possibly induced by the reaction of positively charged chitosan molecules and negatively charged cell membranes (Young et al., 1982; Papineau et al., 1991; Ricke, 2003). However, its inactivation efficacy in seafood seems to be low. Chaiyakosa et al. (2007) found that treatment with 1,000 ppm of chitosan for 120 min caused only less than 0.5 log₁₀ CFU/ml reduction of *V. parahaemoliticus* in naturally contaminated shrimps. Terzi and Gucukoglu (2010) decontaminated *V. parahaemoliticus* in mussel meat with reduction by 1.3 to 2.0 log₁₀ CFU/g using 0.05 to 0.5% chitosan for

5 min. Kong et al. (2010) suggested that the primary antibacterial mechanism of chitosan could be due to electrostatic interactions between its polycationic structure and the predominantly anionic components on the bacterial surface, when the environmental pH is below pK_a of chitosan (the amino group in chitosan has a pK_a value of ~6.5). In our research, the pH of lactic acid solutions containing different levels of chitosan is between 2.33 and 4.59, a condition that may result in domination of protonated chitosan with high density of positive charges readily available to bind to negatively charged surfaces, leading to an interactive effect on bacterial inactivation. We found that chitosan alone had a limited effect on *V. parahaemoliticus* inactivation in our preliminary experiment that only 0.6, 0.6 and 0.8 log₁₀ CFU/g reduction were achieved by washing with 1% chitosan for 10, 20 and 40 min at 110 rpm, respectively. Combination of 2% lactic acid with 1% chitosan for 20 min at 110 rpm reduced the bacterial population by 3.0 log₁₀ CFU/g (Table 2), showing the improved inactivation effect of combined treatments compared to either agent alone (2.2 log₁₀ CFU/g reduction for 2% lactic acid, and 0.6 log₁₀ CFU/g decrease for 1% chitosan as shown in our preliminary experiment) in inactivating *V. parahaemoliticus* in shrimps. The developed response surface model also showed a significant interaction between lactic acid and chitosan for enhanced bacterial inactivation.

The analysis of variance reveals that duration of treatment (10, 20 or 30 min) was not a significant factor ($p > 0.05$) for bacterial reduction, suggesting that 10 min or less was enough for decontamination of *V. parahaemoliticus* in shrimps. This is in agreement with an earlier report showing that dipping fresh raw shrimps into 3% lactic acid for 10, 20 and 30 min could achieve *V. parahaemoliticus* population reduction of 2.6, 2.6, and 2.5 log₁₀ CFU/g, respectively, without significant difference ($p > 0.05$) between different treatment times (Shirazinejad et al., 2010). The reason why this shorter duration was better might be due to the fact that both lactic acid and chitosan are bacteriostatic, not bacteriocidal. Bacteriostatic agents, such as chitosan, are more concentration-dependent (Matthew and Levison, 2004; Kumirska et al., 2011). With regard to model validation, we used bacterial counts observed versus predicted for calculation of A_f and B_f which were initially used for comparison of time-based parameters, such as generation time and growth

rate (Ross, 1996). This approach has been used in several recent publications (Bover-Cid et al., 2012; Geysen et al., 2005; Koseki and Yamamoto, 2007; Skandamis and Nychas, 2000) showing that bacterial count was the result of time-based parameters (growth rate, inactivation rate, generation time, etc.) given by the secondary models, which were integrated into the primary model to generate an overall model.

The regulations for the control of *V. parahaemoliticus* in seafood processing vary in different countries. The Codex Committee on Fish and Fishery Products (WHO/FAO, 2007) proposed that the risk reduction could be estimated when the total number of *V. parahaemoliticus* or the number of pathogenic *V. parahaemoliticus* ranges from absence in 25 g to 1,000 CFU or MPN per gram in mollusks. According to the *V. parahaemoliticus* control plan established by the U.S. Food and Drug Administration (FDA), the control measure used in the post-harvest processing should achieve 2 to 3 log reduction of total *V. parahaemoliticus* for oysters from different coastal areas (FDA, 2011a). With the developed model in this study, the estimated bacterial reduction of 4.5 log₁₀ CFU/g could be achieved under optimized treatment conditions (3% lactic acid, 1.6% chitosan, and rotational rate at 110 rpm), which meets the FDA guideline. The simplified response surface model presented in this study has the capability to predict the decontamination effect of lactic acid and chitosan on *V. parahaemoliticus* in shrimps. The combination of lactic acid and chitosan could be potentially used to mitigate the risk of *V. parahaemoliticus* load in shrimps or possibly other seafood products in seafood processing without causing adverse effects on quality attributes.

Acknowledgments

This research was supported by Zhejiang University. The authors thank Zhoushan Haizhige Seafood Co., Ltd. for providing the information on shrimp processing.

References

- Alakomi, H.L., Skyttä, E., Saarela, M., Sandholm, T.M., Kala, K.L., Helander, I.M., 2000. Lactic acid permeabilizes gram-negative bacteria by disrupting the outer membrane. *Applied and Environmental Microbiology* 66, 2001–2005.
- Alishanhi, A., Aider, M., 2012. Applications of chitosan in the seafood industry and aquaculture: a review. *Food and Bioprocess Technology* 5, 817–830.
- Andrews, L.S., Park, D.L., Chen, Y.P., 2000. Low temperature pasteurization to reduce the risk of *Vibrio* infections from raw shell-stock oysters. *Food Additives and Contaminants* 19, 787–791.
- Andrews, L.S., DeBlanc, S., Veal, C.D., Park, D.L., 2003. Response of *Vibrio parahaemoliticus* O3:K6 to a hot water/cold shock pasteurization process. *Food Additives and Contaminants* 20, 331–334.
- Bas, D., Boyaci, I.H., 2007. Modeling and optimization I: usability of response surface methodology. *Journal of Food Engineering* 78, 836–845.
- Baumann, P., Schubert, R.H.W., 1984. Family II. *Vibrionn16.1(o)dttaof*

- Ross, T., 1996. Indices for performance evaluation of predictive models in food microbiology. *Journal of Applied Bacteriology* 81, 501–508.
- Shirazinejad, A., Ismail, N., Bhat, R., 2010. Lactic acid as a potential decontaminant of selected foodborne pathogenic bacteria in shrimp (*Penaeus merguensis* de Man). *Foodborne Pathogens and Disease* 7, 1531–1536.
- Skandamis, P.N., Nychas, G.J.E., 2000. Development and evaluation of a model predicting the survival of *Escherichia coli* O157:H7 NCTC 12900 in homemade eggplant salad at various temperatures, pHs, and oregano essential oil concentrations. *Applied and Environmental Microbiology* 66, 1646–1653.
- Terzi, G., Gucukoglu, A., 2010. Effects of lactic acid and chitosan on the survival of *V. parahaemoliticus* in mussel samples. *Journal of Animal and Veterinary Advances* 9 (6), 990–994.
- Venkitanarayanan, K.S., Lin, C.-M., Bailey, H., Doyle, M.P., 2002. Inactivation of *Escherichia coli* O157:H7, *Salmonella Enteritidis*, and *Listeria monocytogenes* on apples, oranges, and tomatoes by lactic acid with hydrogen peroxide. *Journal of Food Protection* 65, 100–105.
- Whiting, R.C., Buchanan, R.L., 1994. Microbial modeling. *Food Technology* 48, 113–120.
- WHO/FAO, 2007. Report of the thirty-eighth session of the Codex Committee on food hygiene. Joint FAO/WHO Food Standards Programme. Rome, Italy. (21 pp.) Available at: http://www.codexalimentarius.net/input/download/report/671/al30_13e.pdf.
- Yang, Z., Jiao, X., Zhou, X., Cao, G., Fang, W., Gu, R., 2008. Isolation and molecular characterization of *Vibrio parahaemoliticus* from fresh, low-temperature preserved, dried, and salted seafood products in two coastal areas of eastern China. *International Journal of Food Microbiology* 125, 279–285.
- Yang, Z.Q., Jiao, X.A., Li, P., Pan, Z.M., Huang, J.L., Gu, R.X., Fang, W.M., Chao, G.X., 2009. Predictive model of *Vibrio parahaemoliticus* growth and survival on salmon meat as a function of temperature. *Food Microbiology* 26, 606–614.
- Ye, M., Neetao, H., Chen, H., 2008. Effectiveness of chitosan coated plastic films incorporating antimicrobials in inhibition of *Listeria monocytogenes* on cold-smoked salmon. *International Journal of Food Microbiology* 127, 235–240.
- Young, D.H., Kohle, H., Kauss, H., 1982. Effect of chitosan on membrane permeability of suspension cultured *G. cine max* and *Phaseolus vulgaris* cells. *Plant Physiology* 70, 1449–1454.
- Zarei, M., Borujeni, M.P., Jamnejad, A., Khezzadeh, M., 2012. Seasonal prevalence of *Vibrio* species in retail shrimps with an emphasis on *Vibrio parahaemoliticus*. *Food Control* 25, 107–109.
- Zhang, J.Y., Mei, L.L., Zhu, M., Zhang, Y.J., Pan, X.X., Shi, Y.S., Zheng, G.Z., 2007. Quantitative inspection and analysis of *Vibrio parahaemoliticus* contamination in 301 seafoods. *Chinese Journal of Health Laboratory Technology* 17, 509–510.