Journal of Aquatic Food Product Technology

ISSN: 1049-8850 (Print) 1547-0636 (Online) Journal homepage: http://www.tandfonline.com/loi/wafp20

Pasteurizing Cold Smoked Salmon (Oncorhynchus nerka): Thermal Inactivation Kinetics of Listeria monocytogenes and Listeria innocua

Yage Shi, Juming Tang, Tianli Yue, Barbara Rasco & Shaojin Wang

To cite this article: Yage Shi, Juming Tang, Tianli Yue, Barbara Rasco & Shaojin Wang (2015) Pasteurizing Cold Smoked Salmon (Oncorhynchus nerka): Thermal Inactivation Kinetics of Listeria monocytogenes and Listeria innocua, Journal of Aquatic Food Product Technology, 24:7, 712-722, DOI: 10.1080/10498850.2013.808303

To link to this article: http://dx.doi.org/10.1080/10498850.2013.808303

Accepted author version posted online: 14 May 2014.
Published online: 14 May 2014.

Journal of Aquatic Food Product Technology, 24:712–722, 2015

ISSN: 1049-8850 print/1547-0636 online
DOI: 10.1080/10498850.2013.808303

Pasteurizing Cold Smoked Salmon (*Oncorhynchus nerka*): Thermal Inactivation Kinetics of *Listeria monocytogenes* and *Listeria innocua*

Yage Shi,[1,2] Juming Tang,[2] Tianli Yue,[1] Barbara Rasco,[3] and Shaojin Wang[2,4]

[1]*College of Food Science and Engineering, Northwest A&F University, Yangling, Shaanxi, China*
[2]*Department of Biological Systems Engineering, Washington State University, Pullman, Washington, USA*
[3]*School of Food Science, Washington State University, Pullman, Washington, USA*
[4]*College of Mechanical and Electronic Engineering, Northwest A&F University, Yangling, Shaanxi, China*

Listeria monocytogenes results in potential food safety concerns in ready-to-eat refrigerated products. Cold-smoked salmon is a food considered to be at high risk for *Listeria* spp. contamination for which a mild heat treatment or pasteurization step might be helpful to reduce microbial levels. Cold-smoked salmon was inoculated with a cocktail of four strains (*L. monocytogenes*: ATCC19114, 7644, and 19113, and *L. innocua*: ATCC51742) and inactivation curves obtained at 58, 60, 62, 64, and 66°C using capillary tube methods. Inactivation results showed typical log linear trends ($R^2 \geq 0.97$). *D*-values of *L. monocytogenes* and *L. innocua* were 0.3 to 14.1 min at 66 to 58°C, with a *z*-value of 5.2 to 6.5°C and activation energy of 332 to 414 kJ/mol. The nonpathogenic *Listeria innocua* ATCC51742 had comparable *D*- and *z*-values to the three strains of *L. monocytogenes* and thus can be used for validation of pasteurization processes to control *L. monocytogenes* in cold-smoked salmon and potentially other ready-to-eat thermolabile food products.

Keywords: *D*-value, *z*-value, cold-smoked salmon, *Listeria monocytogenes*, Listeria innocua

INTRODUCTION

Cold-smoked salmon is a popular high value food in much of Western and Eastern Europe and the United States and has an expanding market in East Asia and South America. It has a delicate texture, a mild flavor, and relatively high levels of polyunsaturated fatty acids (National Marine Fisheries Service [NMFS], 2011). The common processing includes curing in dry salt or treatment in a conventional salt brine that may contain sugar, spices, or other additives, followed by smoking at ≤ 32°C, resulting in a product with 3–8% water phase salt, corresponding to water activity of 0.95–0.98 and a pH of 5.9–6.3.

Correspondence should be addressed to Shaojin Wang, PhD, College of Mechanical and Electronic Engineering, Northwest A&F University, Yangling, Shaanxi 712100, China. E-mail: shaojinwang@nwsuaf.edu.cn

Listeria spp. are prevalent in the estuarine environment, but direct contamination of the cold-smoked salmon with *Listeria monocytogenes* often occurs in processing plants, during initial handling, brining, slicing, and packaging operations (Buchanan et al., 1997; Food and Drug Administration [FDA], 2003, 2011a, 2011b; World Health Organization [WHO], 2004). *L. monocytogenes* adapts well in this type of food matrix and can survive over a wide range of temperatures, including refrigerated temperatures to 4°C (Shin et al., 2008), pH, and water activity (Huss et al., 2000; Lianou et al., 2006; Ross et al., 2000). Preventive controls include good hygiene during processing (Shin et al., 2008) and proper packaging (Shin et al., 2006), which can reduce the level of contamination of *L. monocytogenes* in cold-smoked salmon. Salt addition alone at a level of 3.5–5% has no inhibitory effect on the growth of this microbe in cured ready-to-eat aquatic foods during storage (Peterson et al., 1993; Shin and Rasco, 2007). Hurdle treatments to control *Listeria* spp. growth on these foods are somewhat effective (Al-Holy et al., 2004b, 2005), although other strategies would be helpful for *Listeria* spp. control. Since this product is usually consumed without further cooking, the presence of *L. monocytogenes* in the cold-smoked salmon may present a food safety risk to susceptible individuals (de Valk et al., 2005).

The incidence of *L. monocytogenes* in smoked fish may be as high as 15% (Ben Embarek, 1994; Guyer and Jemmi, 1991; Heinitz and Johnson, 1998; Jemmi and Stephan, 2006). Awareness of the risk coupled with mandatory implementation of the Hazard Analysis and Critical Control Points (HACCP) in the aquatic foods industry in 1995 has likely reduced the incidence in smoked fish produced in the United States (FDA, 2003). The U.S. Department of Agriculture and FDA implemented a zero tolerance for *L. monocytogenes* in ready-to-eat (RTE) meat products (Muriana et al., 2002; Vail et al., 2012), and postpackaging pasteurization treatments are widely employed for deli meats and similar food items to control *Listeria* spp. Thermal processing can be potentially used to control *L. monocytogenes* in cold-smoked salmon (Suutari and Laakso, 1994; Bremer et al., 2002) and might not substantially affect product sensory characteristics, but there is no published information about the thermal resistance of various *L. monocytogenes* strains in this particular type of product.

To develop effective thermal processes, the inactivation kinetics of the target microorganism must first be developed at various processing temperatures under isothermal conditions (Chung et al., 2007; Jordan et al., 2011). Pathogenic *L. monocytogenes* should not be used to validate a pasteurization process in food processing plants because of critical safety requirements for workers, the product, and the processing environment. Instead, a surrogate microorganism should be used. *L. innocua* is nonpathogenic and has been recommended as a surrogate for *L. monocytogenes* in thermal process validations (Al-Holy et al., 2004a, 2004b; Margolles et al., 2000; Miller et al., 2009b).

Parameters derived from first-order thermal inactivation kinetics for food-borne microorganisms—namely, decimal reduction values (D-values) and z-values—have been used to design effective commercial thermal pasteurization processes in production of RTE meat products (Grosulescu et al., 2011). Other kinetic models, such as Weibull-type and modified Gompertz models, are also used to describe survival curves of *L. monocytogenes* under different thermal treatment conditions (Huang, 2009), taking into account possible deviations from first-order kinetics behaviors, typically, the shoulder and tailing effects in the survival curves for thermal treatments using moderate treatment temperatures (56–66°C; Miller et al., 2011). The thermotolerance of *L. monocytogenes* and *L. innocua* depends upon various factors, such as the food matrix, in this case fat and salt levels, water activity, pH values, and heating methods (Juneja et al., 1998). Huang (2009) reported D-values of *L. monocytogenes* to be around 5.6 and 0.4 min at 57 and 63°C, respectively, in ground beef with z-value of 6.0°C. Those values are comparable to that reported for duck muscle, turkey breast, and chicken breast (Murphy et al., 2003). Li et al. (2011) reported D-values of *L. innocua* in poultry to be 8 and 4 min at 60 and 70°C, respectively. Miller et al. (2009a, 2011) also reported that D-values of *L. innocua* in broth were 7.1 and 0.46 min at 57.5 and 65°C, respectively, with z-value of 6.9°C. Except for work conducted on ikura and sturgeon caviars (Al-Holy et al.,

2004a, 2004b, 2005), which have a similar salt content but higher fat than cold-smoked salmon, there is little information available on thermal inactivation kinetics of *L. monocytogenes* strains in RTE aquatic foods and relative thermal resistance of the potential surrogates that could be used for validation of thermal pasteurization processes under mild heating conditions. Having thermal treatment data for cold-smoked salmon would be important because of the popularity of this food in the world market.

The objectives of this study were to determine *D*- and *z*-values of *L. monocytogenes* during thermal inactivation in RTE cold-smoked salmon, calculate thermal death rate constant and activation energy of *L. monocytogenes*, and compare thermal lethality kinetics of *L. monocytogenes* with those of *L. innocua* in RTE cold-smoked salmon to determine if *L. innocua* would be a suitable microorganism for process validation studies.

MATERIALS AND METHODS

Meat Preparation

Cold-smoked sockeye salmon (*Oncorhynchus nerka*) was purchased from a local market (Pullman, WA, USA) and kept at −20°C prior to the experiments. The salmon sample was thawed at 4°C overnight before thermal treatments. The muscle contained about 67.5% moisture and 2.08% salt (as is basis) and had a water activity of 0.96 and a pH of 5.64.

Bacterial Preparation

Three isolates of *L. monocytogenes* (ATCC19114, 7644, 19113) and one potential surrogate isolate of *L. innocua* (ATCC51742) were obtained from the Food Microbiology Laboratory at Washington State University (Pullman, WA, USA). These four strains were selected since they have been commonly observed in seafood products such as salmon and Sturgeon caviars (Al-Holy et al., 2004a, 2004b; Shin et al., 2007). Each isolate strain was transferred individually onto the slants of *Listeria* PALCAM medium base (Difco Laboratories, Sparks, MD, USA) that was supplemented with a Bacto PALCAM antimicrobic-selective supplement. From each slant, a sterile loop of culture was transferred to 50 mL tryptic soy broth (TSB; Difco, Becton Dickinson, Sparks, MD, USA) plus 1% yeast extract in a 250 mL screw-cap dilution bottle and then incubated at 37°C for 24 h. Cells in the stationary phase were used for the thermal inactivation experiments, because this phase is more resistant than those in the active phase of exponential growth (Carlier et al., 1996). *Listeria* cells were harvested by centrifugation at 25,000 × *g* for 30 min and washed in an equal volume of sterile 0.1% peptone water. The supernatant was removed after centrifugation. The pellets were resuspended in 0.1% peptone water (pH 7.2 ± 0.2; Difco) and centrifuged at 25,000 × *g* for 30 min again. This washing procedure was repeated three times to remove residual TSB. The pellet was resuspended in 0.1% peptone water, corresponding to approximately 10^8–10^9 CFU/mL.

Inoculation

Ten grams of smoked salmon (homogenized aseptically) was placed aseptically into a sterile Petri dish and air-dried for 10 min in a biosafety hood with a fan to remove surface moisture, then mixed with 100 μl culture (approximately 10^8–10^9 CFU/mL). The inoculation samples were ground and mixed using a coffee grinder (ID557, Mr. Coffee, Guangzhou, China) for 2 min and followed by a manual mixing to further homogenize the salmon samples. The inoculation level of *L. monocytogenes* and *L. innocua* varied from 10^6 to 10^8 CFU/g.

Each of the inoculated samples was kept at 4°C for 30 min to allow bacterial cells to adhere onto fish tissue. About 0.2 g inoculated sample was carefully and aseptically injected into a glass capillary

tube with an inner diameter of 1.8 mm and an outer diameter of 3 mm (Corning, Inc., Corning, NY, USA), using a sterilized syringe with an 18-gauge, 15.2-cm pipetting needle (Popper & Sons, Inc., New Hyde Park, NJ, USA). The open end of the tubes was heat sealed about 20 mm above the sample to avoid thermal influence on the microbial survival (Al-Holy et al., 2004a). For each trial, inoculated and unheated controls were prepared, and the initial inoculation level determined.

Heat Treatment

Sets of capillary tubes were submerged completely in a circulated water bath (Model ZD, Grant, Cambridge, UK) preheated to one of five temperatures: 58, 60, 62, 64, and 66°C. These temperatures were selected based on the achieved reduction in similar fish products (Al-Holy et al., 2004a, 2004b, 2005) and acceptable salmon quality (Kong et al., 2007). After the sample center temperature reached within 0.5°C from the set-point, the heating time was started ($t = 0$). Sets of capillary tubes were removed from the water bath at predetermined time intervals from 14 s to 18 min depending upon the temperature, so that an approximate equivalent log reduction was achieved at each temperature. After heating, the capillary tubes were removed from the water bath and cooled immediately in an ice water bath.

The central temperature of the uninoculated homogenized cold-smoked salmon inside the glass capillary tube was measured with a thin precalibrated Type-T thermocouples (THQSS-020u-6, Omega Engineering Inc., Stamford, CT, USA) having an accuracy of ± 0.5°C and an 0.8 s response time. All data were recorded with a data logger (DL2e, Delta-T Devices Ltd., Cambridge, UK) for a time interval of 2 s during heating, holding, and cooling. Since a very small ca. 0.2 g muscle sample was used in this study, care on the sensor insertion and fixing was taken to reduce any experimental errors from the temperature measurements.

Bacterial Enumeration

After cooling, both ends of the capillary tubes were aseptically removed. The heated salmon was flushed out of the capillary tube with 2 mL of sterile 0.1% peptone water. Then, the heat treated samples were 10-fold serially diluted in sterile 0.1% peptone water. Appropriate dilutions were made and then 1 mL of each serially diluted sample was pour-plated with TSA medium in duplicate. Solidified plates were incubated for 2 days at 37°C. The number of colonies was manually counted. All experiments were conducted in duplicate.

D- and *z*-Values

To mathematically describe experimental survival curves of the sampled cultures of *L. monocytogenes*, we attempted to fit the data with different orders of reaction and found the first-order to be the best based on the goodness of fit. The first-order thermal inactivation kinetic model is described as follows (Stumbo, 1973; Huang, 2009):

$$\frac{dN}{dt} = -kN, \tag{1}$$

where N (CFU/g) represents microbial population, t (min) is heating time under an isothermal condition, and k (min^{-1}) is the rate constant. After integration, the above equation can be represented as a plot of the log of the number of surviving cells versus heating time (Stumbo, 1973; Chung et al., 2007; Muriana et al., 2002):

$$\log N = \log N_o - \frac{t}{D}. \tag{2}$$

D-values were obtained from –1/slope of the best-fit line at each temperature with $D = 2.303/k$. Plotting log D-values against temperature is often linear and is commonly referred to as the thermal-death-time (TDT) curve (Holdsworth, 1997).

A z-value was obtained as the temperature increases, resulting in one log reduction in D-value from the TDT curve, that is:

$$z = \frac{T_2 - T_1}{\log D_{T_1} - \log D_{T_2}}, \tag{3}$$

where D_T represents value of D measured at temperature T, and T_1 and T_2 are two different temperatures (°C). The z-value (°C) was obtained from the –1/slope of the regression equation plotting the log D value versus temperature.

Activation Energy

Two independent methods were used to calculate the thermal lethality activation energy (E_a in Joules per mole) of *L. monocytogenes* and *L. innocua* in RTE cold-smoked salmon. E_a is used to determine the sensitivity of pathogens to changes in temperature; higher activation energy refers to higher sensitivity. E_a was calculated using the first method by the following equation:

$$E_a = \frac{2.303 R\, T_{\min} T_{\max}}{z}, \tag{4}$$

where R is the universal gas constant (8.314 J/mol K), and T_{min} and T_{max} are the minimum and maximum absolute temperatures (°K), respectively, of a test range. The second method for calculating E_a was by using the slope of an Arrhenius plot of log k versus the reciprocal of the absolute temperature ($1/T$) as follows:

$$log\, k = log\, k_0 - \frac{E_a}{2.303\, RT}, \tag{5}$$

where k_0 is the reference thermal lethality rate constant (1/min).

Statistical Analysis

Differences in D-values of four different strains subjected to heat treatments were analyzed from two independent duplicate experiments. The mean differences were separated at significance level of $p = 0.05$ (SAS Institute, Cary, NC, USA).

RESULTS AND DISCUSSIONS

Heat Treatments

Figure 1 shows temperature–time histories (core temperatures) for the samples in the capillary tubes at five selected temperatures. Come-up times averaged approximately 14 s for all the test temperatures, after which the samples experienced close to ideal isothermal exposure. This come-up time is similar to that observed in the same capillary tube with mashed potato samples (Chung et al., 2007) and salted cured ikura (Al-Holy et al., 2005). The sample temperature during holding time was

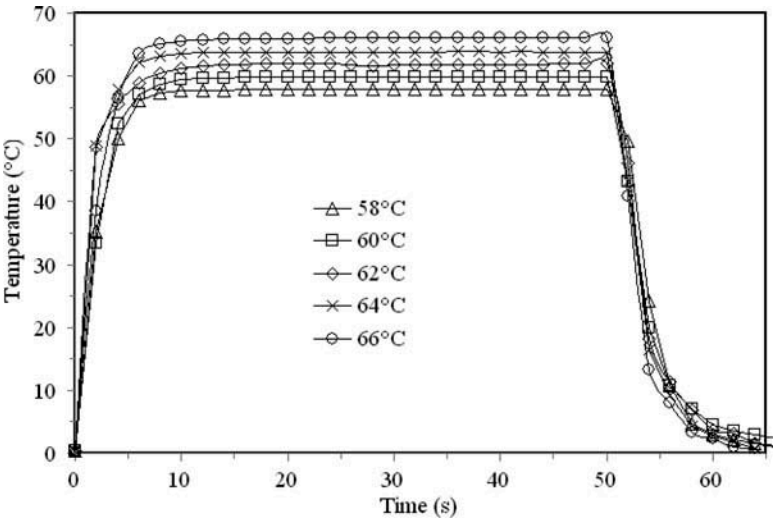

FIGURE 1 Temperature-time history for center (core) temperature of smoked salmon homogenate in capillary tubes heated in hot water bath at the listed set-points. The samples were cooled in ice water.

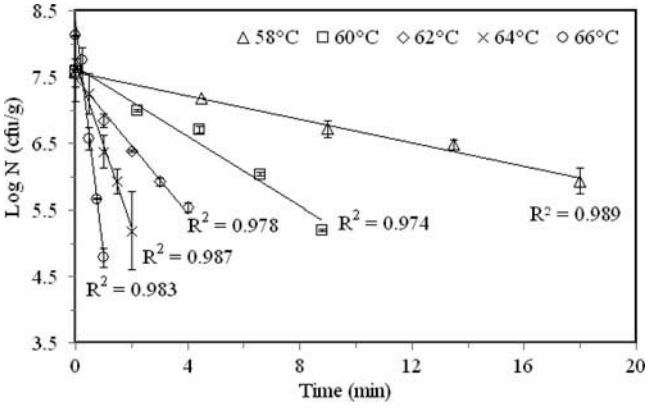

FIGURE 2 Survivor curves of *L. monocytogenes* ATCC7644 in cold-smoked salmon heated at five temperatures.

relatively constant and the cooling process took less than 8 s for core temperatures to drop to less than 10°C. Figure 2 shows typical survival curves of *Listeria monocytogenes* strains ATCC7644 at the five tested temperatures. Similar survival curves of other three strains were also obtained but only *D*-values are shown in Table 1. The survival curves were semi-log linear with high coefficient of determination ($R^2 \geq 0.974$), without showing initial shoulders and tails, which could be caused by instant heating and cooling using the capillary tube (Chung et al., 2007) or TDT cells (Jin et al., 2008). Thus these survival curves can be described using first-order kinetics under isothermal conditions (Chung et al., 2007; Sorqvist, 2003).

D- and *z*-Values

D-values of *L. monocytogenes* and *L. innocua* are summarized in Table 1. As expected, *D*-values of the four strains decreased sharply with increasing temperature from 58 to 66°C. At low

TABLE 1
D- and *z*-values (mean ± standard deviation, $n = 2$) for *Listeria monocytogenes* and *L. innocua* (51742) at five temperatures

	D-values (min)					
ATCC	*58°C*	*60°C*	*62°C*	*64°C*	*66°C*	z-*value (°C)*
19113	14.09 ± 0.26a*	3.76 ± 0.09a	2.27 ± 0.07a	1.06 ± 0.03a	0.64 ± 0.06a	6.18 ± 0.13ab
7644	11.33 ± 0.12b	3.82 ± 0.04a	1.96 ± 0.07a	0.81 ± 0.01b	0.30 ± 0.02b	5.23 ± 0.08c
19114	8.10 ± 0.11d	3.83 ± 0.13ab	2.34 ± 0.15a	1.12 ± 0.02a	0.43 ± 0.01ab	6.45 ± 0.01a
51742	9.68 ± 0.06c	4.24 ± 0.04b	2.13 ± 0.07a	1.08 ± 0.08ab	0.44 ± 0.02a	6.06 ± 0.01b

*Within each column, means followed by different letters are significantly different ($p < 0.05$).

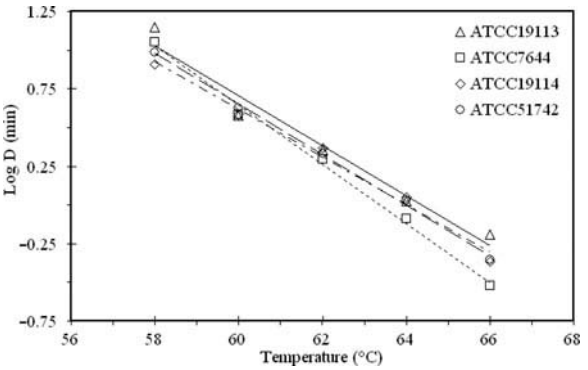

FIGURE 3 Thermal death time curves for *L. monocytogenes* and *L. innocua* in cold-smoked salmon homogenates.

treatment temperatures (58 or 60°C), *L. innocua* ATCC51742 was significantly less heat resistant than *L. monocytogenes* ($p < 0.05$). But at high temperatures (> 60°C), *D*-values of *L. innocua* ATCC51742 were statistically similar to or larger than those of *L. monocytogenes* ($p < 0.05$), suggesting that *L. innocua* ATCC51742 can be used as a conservative surrogate of *L. monocytogenes* in cold-smoked salmon for validating thermal treatments at temperatures of 60°C or higher, but may not be reliable at lower temperatures. Similar heat resistance has been observed by Al-Holy et al. (2004a, 2004b, 2005) for fish roe products at similar salt content and by others for aquatic foods (Ben Embarek, 1994; Ben Embarek and Huss, 1993; Huss et al., 2000).

Figure 3 shows relationships between *D*-values and heating temperatures for the tested strains. The *z*-values were obtained by linear regression of the log *D* against temperature with $R^2 =$ 0.965–0.998. The *z*-values of *L. innocua* ATCC51742 and ATCC 19113 were significantly larger than those of ATCC 7644 but smaller than those of ATCC 19114 ($p < 0.05$; Table 1). The average *z*-values varied between 5.2 and 6.2°C, which are comparable to other stains of *L. monocytogenes* tested in ground beef, duck muscle, turkey breast, and chicken breast (Farber and Peterkin, 1991; Huang, 2009; Murphy et al., 2003). The heat resistant difference between this study and others is a result of differences in pH and salt content and to a lesser extent the heating method used (Doyle et al., 2001; Ghazala, 1998; Juneja et al., 1998).

Many factors that may influence the thermal resistant data must be taken into account for comparisons. *D*-values for *L. monocytogenes* in surimi-based imitation crabmeat are 2.1 min at 62°C and 0.4 min at 66°C for stationary-phase pathogens that were heated in thin plastic pouches (Mazzotta, 2001), a material that has significantly less fat and a much lower salt content than cold-smoked

salmon. A *D*-value of 4.2–4.5 min at 60°C for two strains of *L. monocytogenes* in vacuum packaged sous-vide cooked salmon fillet was reported by Ben Embarek and Huss (1993), and *D*-values of 3.0, 0.8, and 0.4 min at 60, 63, and 65°C, respectively, were obtained using a novel aluminum tube for salmon caviar with similar ionic strength to some cold-smoked salmon but a higher fat content (Al-Holy et al., 2004a). Bremer and Osborne (1995) observed *D*-values of *L. monocytogenes* in green shell mussels to be 16.25, 5.49, and 1.85 min at 58, 60, and 62°C, respectively, a material that is substantially different biologically from salmon muscle. Muriana et al. (2002) reported $D_{62.8} = 6.9$ min and $D_{65.6} = 1.2$ min for RTE smoked turkey, $D_{62.8} = 1.6$ min and $D_{65.6} = 0.9$ min for roast beef, and $D_{62.8} = 1.13$ and $D_{65.6} = 0.49$ min for smoked ham, respectively. These studies reported a *z*-value for RTE smoked turkey, roast beef, and smoked ham of 5.1, 5.7, and 7.9°C, respectively. Several studies on the thermal resistance of *L. monocytogenes* in meat products show that *z*-values range from 4.6 to 4.7 (Carlier at al., 1996). Although a wide range of *D*- and *z*-values of *L. monocytogenes* is reported in the literature, the *D*-value of 2 min at 62°C and an average *z*-value of 6°C seem to be typical (Augustin, 1996; Fairchild and Foegeding, 1993; Farber, 1989; Mackey and Bratchell, 1989).

Thermal Death Rate Constant and Activation Energy

Figure 4 shows an Arrhenius plot for temperature effects on thermal lethality rate constant both for *L. monocytogenes* and *L. innocua* in cold-smoked salmon samples. The log *k* decreased lineally with the reciprocal of the absolute temperature for all four strains. The reciprocal of the slopes could be used to calculate the activation energy using Equation (5).

Table 2 compares the activation energies for *L. monocytogenes* and *L. innocua* estimated by both the TDT curve and the *k*-*T* curve methods. The differences in activation energies between TDT and *k*-*T* curves were small both for *L. monocytogenes* (< 3.5%) and *L. innocua* (1.2%). The activation energies for all four strains were similar, which was expected based on the similar slopes of TDT curves in Figure 3. The ATCC 7644 strain showed the greatest differences in activation energy from the other tested strains. The observed level of activation energy was in good agreement with those reported for controlling *L. monocytogenes* in ground chicken breast (381 kJ/mol; Murphy et al., 2000) and *Listeria* in chicken patties (352 kJ/mol; Murphy et al., 2001) and soymilk (305 kJ/mol; Igyor et al., 2012).

With good hygiene practices in processing plants, the numbers of *L. monocytogene*s have found to be low in cold-smoked salmon—e.g., 0.3 and 34 cells/g reported in Eklund et al. (1995). Thus,

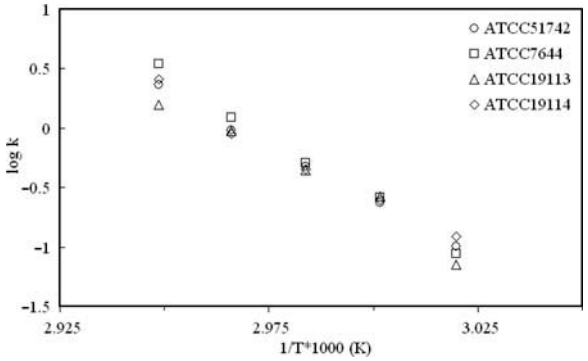

FIGURE 4 Arrhenius plot for temperature effects on the thermal lethality rate constant for *L. monocytogenes* and *L. innocua* in cold-smoked salmon.

TABLE 2
Activation energy for *Listeria* calculated from thermal death time (TDT) curves (Equation 4) and *k-T* curves (Equation 5)

	Activation energy (kJ/mol)	
ATCC	*TDT curve*	*k-T curve*
19113	348.0	349.6
7644	413.6	416.4
19114	331.9	343.4
51742	352.5	356.6

a typical 6 log reduction of the microorganism may be used as the criteria to design a thermal pasteurization process. Based on Table 1, a 6 log thermal process would require 7 min full exposure at 64°C or 4 min at 66°C. Pasteurizing under these conditions is likely to have a significant impact on the sensory characteristics that cold-smoked salmon is prized for. Further studies are needed to evaluate the influence of such thermal processes on consumer acceptance of thermally treated products.

CONCLUSIONS

The isothermal heating was achieved in cold-smoked salmon homogenates using a capillary tube method with a short come-up time of 14 s. The four selected strains (ATCC19114, 7644, 19113, and 51742) of *L. monocytogenes* and *L. innocua* demonstrated typical first-order inactivation characteristics under isothermal test conditions. The *D*-values of *L. monocytogenes* and *L. innocua* decreased sharply with increasing temperature from 58 to 66°C. The *z*-values varied between 5.2 and 6.2°C for *Listeria* strains. The activation energies for *L. monocytogenes* and *L. innocua* were reliably estimated by both the TDT curve and the *k-T* curve methods, resulting in small differences of below 3.5%. Although the relative heat resistance demonstrated the potential of a pasteurizing process to protect the product from *Listeria*, more studies are required to determine the impact of such a process on the sensory characteristics of the product. Any large-scale validation studies using *L. innocua* must be conducted at temperatures greater than 60°C for pasteurization of cold-smoked salmon products.

ACKNOWLEDGMENTS

This research was conducted while the first author was a visiting scholar at the Department of Biological Systems Engineering, Washington State University (WSU).

FUNDING

The project was partially supported by grants from WSU Agricultural Research Center and provided by the USDA-NIFA-AFRI (2011-68003-20096).

REFERENCES

Al-Holy, M., Lin, M., and Rasco, B.A. 2005. Inactivation of *Listeria monocytogenes* in sturgeon (*Acipenser transmontanus*) caviar using a combination of nisin with chemical antimicrobials or moderate heat. J. Food Prot. 68(3): 512–520.

Al-Holy, M., Quinde, Z., Guan, D., Tang, J., and Rasco, B. 2004a. Thermal inactivation of *Listeria innocua* in salmon (*Oncorhynchus keta*) caviar using conventional glass and novel aluminum thermal-death-time tubes. J. Food Prot. 67(2): 383–386.

Al-Holy, M., Ruiter, J., Lin, M., Kang, D. H., and Rasco, B. 2004b. Inactivation of *Listeria innocua* in nisin treated salmon (*Oncorhynchus keta*) and sturgeon (*Acipenser transmontanus*) in caviar treated by radio frequency (RF). J. Food Prot. 67(9): 1848–8154.

Augustin, J. C. 1996. Résistance de *Listeria monocytogenes* aux traitements physiques. Pathologie-biologie 44(9): 790–807.

Ben Embarek, P. K. 1994. Presence, detection and growth of *Listeria monocytogenes* in seafoods: A review. Int. J. Food Microbiol. 23(1): 17–34.

Ben Embarek, P. K., and Huss, H. H. 1993. Heat resistance of *Listeria monocytogenes* in vacuum packaged pasteurized fish fillets. Int. J. Food Microbiol. 20(2): 85–95.

Bremer, P. J., and Osborne, C. M. 1995. Thermal-death times of *Listeria monocytogenes* in green shell mussels (*Perna canaliculus*) prepared for hot smoking. J. Food Prot. 58(6): 604–608.

Bremer, P. J., Monk, I., Osborne, C. M., Hills, S., and Butler, R. 2002. Development of a steam treatment to eliminate *Listeria monocytogenes* from King salmon (*Oncorhynchus tshawytscha*). J. Food Sci. 67(6): 2282–2287.

Buchanan, R. L., Damert, W. G., Whiting, R. C., and van Schothorst, M. 1997. Use of epidemiologic and food survey data to estimate a purposefully conservative dose-response relationship for *Listeria monocytogenes* levels and incidence of listeriosis. J. Food Prot. 60(8): 918–922.

Carlier, V., Augustin, J. C., and Rozier, J. 1996. Heat resistance of *Listeria monocytogenes* (Phagovar 2389/2425/3274/2671/47/108/340): D- and z-values in ham. J. Food Prot. 59(6): 588–591.

Chung, H., Wang, S., and Tang, J. 2007. Influence of heat transfer in test tubes on measured thermal inactivation parameters for *Escherichia coli*. J. Food Prot. 70(4): 851–859.

de Valk, H., Jacquet, C., Goulet, V., Vaillant, V., Perra, A., Simon, F., Desenclos, J. C., and Martin, P. 2005. Surveillance of *Listeria* infections in Europe. Euro Surveill: Eur. Commun. Dis. Bull. 10(10): 251–255.

Doyle, M. E., Mazzotta, A. S., Wang, T., Wiseman, D. W., and Scott, V. N. 2001. Heat resistance of *Listeria monocytogenes*. J. Food Prot. 64(3): 410–429.

Eklund, M. W., Poysky, F. T., Paranjpye, R. N., Lashbrook, L. C., Peterson, M. E., and Pelroy, G. A. 1995. Incidence and sources of *Listeria monocytogenes* in cold-smoked fishery products and processing plants. J. Food Prot. 58(5): 502–508.

Fairchild, T. M., and Foegeding, P. M. 1993. A proposed nonpathogenic biological indicator for thermal inactivation of *Listeria monocytogenes*. Appl. Environ. Microbiol. 59(4): 1247–1250.

Farber, J. M. (1989). Thermal resistance of *Listeria monocytogenes* in foods. Int. J. Food Microbiol. 8(3): 285–291.

Farber, J. M., and Peterkin, P. I. 1991. *Listeria monocytogenes*, a food-borne pathogen. Microbiol. Rev. 55(3): 476–511.

FDA. 2003. Quantitative assessment of relative risk to public health from foodborne *Listeria monocytogenes* among selected categories of ready-to-eat foods. Retrieved from: http://purl.access.gpo.gov/GPO/LPS110381

FDA. 2011a. Fish and Fisheries Products Hazards and Control Guide (4th ed.). Retrieved from http://www.fda.gov/downloads/Food/GuidanceRegulation/UCM251970.pdf

FDA. 2011b. Processing Parameters Needed to Control Pathogens in Cold-Smoked Fish. Retrieved from http://www.fda.gov/Food/FoodScienceResearch/SafePracticesforFoodProcesses/ucm092182.htm

Ghazala, S. 1998. Sous Vide and Cook-Chill Processing for the Food Industry. Gaithersburg, MD: Aspen Publishers.

Grosulescu, C., Juneja, V. K., and Ravishankar, S. 2011. Effects and interactions of lactate, sodium discetate, and pediocin on the thermal inactivation of starved *Listeria monocytogenes* on bologna. Food Microbiol. 28: 440–446.

Guyer, S., and Jemmi, T. 1991. Behavior of *Listeria monocytogenes* during fabrication and storage of experimentally contaminated smoked salmon. Appl. Environ. Microbiol. 57(5): 1523–1527.

Heinitz, M. L., and Johnson, J. M. 1998. The incidence of *Listeria* spp., *Salmonella* spp., and *Clostridium botulinum* in smoked fish and shellfish. J. Food Prot. 61(3): 318–323.

Holdsworth, S. D. 1997. Thermal Processing of Packaged Foods. New York, NY: Blackie Academic & Professional.

Huang, L. 2009. Thermal inactivation of *Listeria monocytogenes* in ground beef under isothermal and dynamic temperature conditions. J. Food Eng. 90: 380–387.

Huss, H. H., Jørgensen, L. V., and Vogel, B. F. 2000. Control options for *Listeria monocytogenes* in seafoods. Int. J. Food Microbiol. 62(3): 267–274.

Igyor, M. A., Ariahu, C. C., and Inyang, C. U. 2012. Thermodynamics of heat inactivation of *Listeria monocytogenes* in soymilk of varying pH and sugar values. J. Food Technol. 10(2): 17–23.

Jemmi, T., and Stephan, R. 2006. *Listeria monocytogenes*: Food-borne pathogen and hygiene indicator. Rev. Sci. Tech. Oie. 25(2): 571–580.

Jin, T., Zhang, H., Boyd, G., and Tang, J. 2008. Thermal resistance of *Salmonella enteritidis* and *Escherichia coli* K12 in liquid egg determined by thermal-death-time disks. J. Food Eng. 84: 608–614.

Jordan, J. S., Gurtler, J. B., Marks, H. M., Jones, D. R., and Shaw, W. K. 2011. A mathematical model of inactivation kinetics for a four-strain composite of *Salmonella enteritidis* and *oranienburg* in commercial liquid egg yolk. Food Microbiol. 28(1): 67–75.

Juneja, V. K., Foglia, T. A., and Marmer, B. S. 1998. Heat resistance and fatty acid composition of *Listeria monocytogenes*: Effect of pH, acidulant, and growth temperature. J. Food Prot. 61(6): 683–687.

Kong, F., Tang, J., Rasco, B., and Crapo, C. 2007. Kinetics of salmon quality changes during thermal processing. J. Food Eng. 83: 510–520.

Li, M., Pradhan, A., Cooney, L., Mauromoustakos, A., Crandall, P., Slavik, M., and Li, Y. B. 2011. A predictive model for the inactivation of *Listeria innocua* in cooked poultry products during post-package pasteurization. J. Food Prot. 74(8): 1261–1267.

Lianou, A., Stopforth, J. D. Yoon, Y., Wiedmann, M., and Sofos, J. N. 2006. Growth and stress resistance variation in culture broth among *Listeria monocytogenes* strains of various serotypes and origins. J. Food Prot. 69(11): 2640–2647.

Mackey, B. M., and Bratchell, N. 1989. The heat resistance of *Listeria monocytogenes*. Lett. Appl. Microbiol. 9(3): 89–94.

Margolles, A., Mayo, B., and Reyes-Gavilan, C. G. 2000. Phenotypic characterization of *Listeria monocytogenes* and *Listeria innocua* strains isolated from shortripened cheeses. Food Microbiol. 17: 461–467.

Mazzotta, A. S. 2001. Thermal inactivation of stationary phase and salt-adapted *Listeria monocytogenes* during postprocess pasteurization of surimi-based imitation crabmet. J. Food Prot. 64(4): 483–485.

Miller, F. A., Gil, M. M., Brandao, T. R. S., Teixeira, P., and Silva, C. L. M. 2009a. Sigmoidal thermal inactivation kinetics of *Listeria innocua* in broth: Influence of strain and growth phase. Food Control 20: 1151–1157.

Miller, F. A., Ramos, B., Gil, M. M., Brandao, T. R. S., Teixeira, P., and Silva, C. L. M. 2009b. Influence of pH, type of acid and recovery media on the thermal inactivation of *Listeria innocua*. Int. J. Food Microbiol. 133: 121–128.

Miller, F. A., Ramos, B. F., Gil, M. M., Brandao, T. R. S., Teixeira, P., and Silva, C. L. M. 2011. Heat inactivation of *Listeria innocua* in broth and food products under non-isothermal conditions. Food Control 22: 20–26.

Muriana, P. M., Quimby, W., Davidson, C. A., and Grooms, J. 2002. Postpackage pasteurization of ready-to-eat deli meats by submersion heating for reduction of *Listeria monocytogenes*. J. Food Prot. 65(6): 963–969.

Murphy, R. Y., Duncan, L. K., Beard, B. L., and Driscoll, K. H. 2003. D and z values of *Salmonella, Listeria innocua*, and *Listeria monocytogenes* in fully cooked poultry products. J. Food Sci. 68(4): 1443–1447.

Murphy, R. Y., Marks, B. P., Johnson, E. R., and Johnson, M. G. 2000. Thermal inactivation kinetics of *Salmonella* and *Listeria* in ground chicken breast meat and liquid medium. J. Food Sci. 65(4): 706–710.

Murphy, R. Y., Johnson, E. R., Marks, B. P., Johnson, M. G., and Marcy, J. A. 2001. Thermal inactivation of *Salmonella senftenberg* and *Listeria innocua* in ground chicken breast processed in an air convection oven. Poultry Sci. 80: 515–521.

National Marine Fisheries Service. 2011. Report on the Status of U.S. Fisheries for 2010. Silver Spring, MD: Author.

Peterson, M. E., Pelroy, G. A., Paranjpye, R., Almond, J., and Eklund, M. W. 1993. Parameters for control *Listeria monocytogenes* in smoked fishery products: Sodium chloride and packaging method. J. Food Prot. 56(11): 938–943

Ross, T., Dalgaard, P., and Tienungoon, S. 2000. Predictive modeling of the growth and survival of *Listeria* in fishery products. Int. J. Food Microbiol. 62(3): 231–245.

Shin, J. H., Kang, D. H., and Rasco, B. A. 2006. Survival and growth of *Listeria monocytogenes* in MAP packaged fresh rainbow trout. World Aquac. 37(3): 6–10.

Shin, J. H., Kang, D. H., and Rasco, B. 2008. Effect of different packaging methods and storage temperatures on the growth of *Listeria monocyteoges* in raw and hot smoked rainbow trout (*Oncorhynchus mykiss*). J. Aquat. Food Prod. T. 17(2): 137–155.

Shin, J. H., and Rasco, B. A. 2007. Effect of water phase salt content and storage temperature on *Listeria monocytogenes* survival in chum salmon (*Oncorhynchus keta*) roe and caviar (*ikura*). J. Food Sci. 72(5): M160–M165.

Sorqvist, S. 2003. Heat resistance in liquids of *Enterococcus* spp., *Listeria* spp., *Escherichia coli, Yersinia enterocolitica, Salmonella* spp., and *Campylobacter* spp. Acta Vet. Scand. 44: 1–19.

Stumbo, C. R. 1973. Thermobacteriology in Food Processing. San Diego, CA: Academic Press.

Suutari, M., and Laakso, S. 1994. Microbial fatty acids and thermal adaptation. Crit. Rev. Microbiol. 20(4): 285–328.

Vail, K. M., McMullen, L. M., and Jones, T. H. 2012. Growth and filamentation of cold-adapted, log-phase *Listeria monocytogenes* exposed to salt, acid, or alkali stress at 3 degrees C. J. Food Prot. 75(12): 2142–2150.

WHO. 2004. Risk assessment of *Listeria monocytogenes* in ready-to-eat foods technical report. Geneva, Switzerland: Author.