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Effect of temperature and salt on thermal inactivation of *Listeria monocytogenes* in salmon roe☆

Changcheng Li [a], Lihan Huang [b, *], Cheng-An Hwang [b]

[a] School of Food Science, Fujian Agriculture and Forestry University, Fuzhou, 350001, China
[b] U.S. Department of Agriculture, Agricultural Research Service, Eastern Regional Research Center, 600 E. Mermaid Lane, Wyndmoor, PA 19038, USA

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ABSTRACT

Listeria monocytogenes is a potentially fatal foodborne pathogen that can be found in ready-to-eat seafood products, such as fresh salmon roe. Once contaminated, salmon roe must be decontaminated prior to human consumption. This study was conducted to determine the thermal inactivation kinetics of *L. monocytogenes* in raw salmon roe as affected by bacterial strain, temperature, and salt concentration. Three different strains of *L. monocytogenes*, including serotype 4b (F2365), 1/2b (F4260), and 1/2a (V7), were individually inoculated to salmon roe supplemented with salt (0–4.5%), and heated under different temperatures (57.5–65.0 °C) to evaluate the survival of the bacterium during heating and determine the D-values. Results showed that the thermal resistance (log D) of *L. monocytogenes* was significantly affected by bacterial strain, temperature, and salt and by their interactive effects, with strain F2365 being the most heat-resistant among all three strains tested. Salt added to salmon roe significantly increased the thermal resistance of the bacteria. For *L. monocytogenes* F2365, the z value of the bacterium in salmon roe was 5.99 °C, and its heat resistance increased with the level of salt in a linear manner. The results of kinetic analysis and the models obtained in this study may be used by the seafood industry to develop proper thermal processes to eliminate *L. monocytogenes* in raw salmon roe and to ensure microbial safety and prevent foodborne illness.

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1. Introduction

Caviars are lightly salted and preserved fish roe products with high nutritional and economic values. While the most widely recognized and valued caviar is made from sturgeon, salmon caviar is one of the most popular products because of its relative abundance, attractive red color, and distinctive taste. The U.S. is one of the biggest producers of salmon roe and exports 90% of the products to Asia and Europe (Bledsoe, Bledsoe, & Rasco, 2003). The U.S. exported 11,738 metric tons of salmon caviar and roe, worth of $163 million in 2011 (NOAA, 2011) and 15,307 metric tons, worth of $255 million in 2013 (NOAA, 2014).

Grown in the ocean, fish may carry a variety of spoilage and pathogenic microorganisms, such as *Vibrio parahaemolyticus*, *Escherichia coli*, *Listeria monocytogenes*, and *Salmonella* spp. (Novotny, Dvorska, Lorencova, Beran, & Pavlik, 2004). While live fish flesh and eggs are sterile, the food processing environment is not sterile, leading to cross-contamination in the products. *L. monocytogenes* is frequently found in fish processing environments (Lunestad, Truong, & Lindstedt, 2013) and has been found in various fish roe products (Jami, Ghanbari, Zunabovic, Domig, & Kneifel, 2014). Himelbloom and Crapo (1998) reported that aerobic plate counts (APCs) greater than 10^7 CFU/g and coliform counts higher than 10^3 CFU/g were detected in Alaska salmon caviar. Miya, Takahashi, Ishikawa, Fujii, and Kimura (2010) reported that *L. monocytogenes* was detected in up to 11.4% of cod roe (tarako) samples and 10.0% of salmon roe (ikura) samples tested. Other pathogens, such as *V. parahaemolyticus*, *Salmonella* spp., *Staphylococcus aureus*, *E. coli*, *Shigella* spp., and *Clostridium perfringens*, were found in grey mullet roe (Voidarou et al., 2011). According to Bledsoe et al. (2003), salmon roe is low in acidity (pH 5.2–6.7) and high in water activity (0.96–0.98), and is capable of supporting the growth of microorganisms. Therefore, fish roe products are a potential vehicle for transmission of foodborne pathogens such as *L. monocytogenes*.

L. monocytogenes is a Gram-positive, non-spore forming

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* Corresponding author.
E-mail address: lihan.huang@ars.usda.gov (L. Huang).

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bacterium that is ubiquitously distributed in the environment. It has been isolated from water, soil, food products, and the intestinal tracts of humans and animals (ICMSF, 1996). This bacterium is capable of growing under adverse environmental conditions, such as low temperature (4 °C), high salt concentration (>10%), and low pH (McClure, Roberts, & Otto Oguru, 1989). Consumption of food contaminated with *L. monocytogenes* can lead to listeriosis, which is rare but with a relatively high mortality rate. The Centers for Disease Control and Prevention (CDC) estimated that *L. monocytogenes* causes approximately 1600 illnesses, 1500 hospitalizations, and 260 deaths annually in the U.S. (Scallan et al., 2011). To reduce foodborne listeriosis and protect public health, a "zero-tolerance" policy for this pathogen in cooked and RTE foods has been implemented in the U.S. (USDA-FSIS, 2003).

While fish roe products can be eaten raw, they may be inadvertently contaminated with *L. monocytogenes*. Once they are contaminated, an intervention process is needed to inactivate the pathogen. While some emerging nonthermal technologies such as pulsed electric field (Gudmundsson & Hafsteinsson, 2001) have been reported for use to control *L. monocytogenes* in fish roe, the scale-up feasibility and efficacy of these technologies remain uncertain and need further examination. Thermal processing remains a method of choice as an effective intervention strategy to inactivate both pathogenic and spoilage microorganisms in foods, including fish roe products.

Thermal treatment has been found to be suitable for inactivation of *L. monocytogenes* and *Listeria innocua* (a surrogate) in fish roe (Al-Holy, Ruiter, Lin, Kang, & Rasco, 2004b, 2004a; Miettinen, Arvola, & Wirtanen, 2005) and *L. monocytogenes* in rainbow trout roe (Miettinen et al., 2005) containing 2.5% of salt. Generally, fish roe products contain 3.0–4.0% of salt (Craig & Powrie, 1988). The species of salmon and the condition and degree of maturity of the roe can affect the uptake of salt in fish roe (Huang et al., 2001). According to Jorgensen, Stephens, and Knochel (1995), osmotic adaption by exposure to NaCl significantly increases the thermotolerence of *L. monocytogenes* in modified tryptic phosphate broth and minced beef. However, little information is available concerning the effect of different concentrations of salt on the thermal resistance of *L. monocytogenes* in salmon roe. Therefore, the main objective of this study was to investigate the effect of salt on the thermal resistance of three main serotypes of *L. monocytogenes* in salmon roe. The results obtained from this research may help the food industry to design effective thermal treatments for controlling *L. monocytogenes* in salmon roe products.

2. Materials and methods

2.1. Bacterial strains and preparation of inoculum

Three rifampicin-resistant (100 mg/L) *L. monocytogenes* strains, including *L. monocytogenes* serotype 4b (F2365), *L. monocytogenes* serotype 1/2b (F4260), and *L. monocytogenes* serotype 1/2a (V7), were used in this study. The antibiotic-resistant strains of *L. monocytogenes* were obtained from the culture collection of the Eastern Regional Research Center (ERRC) of the USDA Agricultural Research Service (ARS) located in Wyndmoor, PA (Fang & Huang, 2014; Fang, Liu, & Huang, 2013; Li et al., 2016), and were used to differentiate the inoculated *L. monocytogenes* from numerous background bacteria in the salmon roe samples. The cultures were prepared by streaking each strain of the overnight culture onto tryptic soy agar (TSA, BD/Difco Laboratories, Sparks, MD) plates supplemented with 100 mg/L rifampicin (TSA/R, Sigma, R 3501-5G, Sigma-Aldrich Co., MO). To maintain the viability of the cells, the rifampicin-resistant *L. monocytogenes* cultures were regularly propagated and maintained on TSA/R plates and stored at 4 °C.

One day prior to an experiment, a loopful of each strain was individually inoculated into 10 ml brain heart infusion broth (BHI broth, BD/Difco Laboratories) supplemented with 100 mg/L rifampicin and incubated at 37 °C on an orbital shaker with mild agitation (~100 rpm) for approximately 18–20 h. The cultures were harvested by centrifugation (2400 *g*) at 4 °C for 15 min, washed once with 10 ml 0.1% peptone water (PW, BD/Difco Laboratories), re-centrifuged, and re-suspended in 5 ml 0.1% PW. Each culture was used as the working culture directly, containing a final concentration of approximately 10^9 CFU/ml of *L. monocytogenes*.

2.2. Sample preparation and inoculation

Unsalted fresh pink salmon roe was obtained from a seafood company in Alaska. The samples were divided into smaller bags (50 g) and frozen at −80 °C. One night before experiment, a bag of salmon roe was transferred to a refrigerator (4 °C) for thawing. The thawed salmon roe samples (1 ± 0.02 g) were aseptically weighted into sterile filter bags (Whirl-Pak®, 207 ml, 95 mm × 180 mm × 0.08 mm, NASCO, Fort Atkinson, WI). Salmon roe with different salt concentrations was prepared by adding a different volume of a salt solution (25%) into the sample bags, with the final salt concentration adjusted to 0, 1.5, 3, or 4.5%. Each sample bag was individually inoculated with 100 μl of each strain of *L. monocytogenes* culture. The inoculated salmon roe was smashed by hand and gently mixed for at least 2 min, and then flattened with a round bottle to a thin layer (<0.2 mm). To ensure uniform heating, the sample bags were vacuumed to evacuate the internal air and then sealed when the internal pressure reached 2.0 kPa. Uninoculated samples were used as negative controls. The population of *L. monocytogenes* was ca. 7.0–8.5 log CFU/g in the inoculated samples.

2.3. Thermal inactivation

The inoculated samples bags were subjected to submersion heating in a circulating water bath (Neslab RTE17, Thermo Fisher Scientific, Newington, NH) maintained at 57.5, 60, 62.5, or 65 °C. The samples were fully submerged in hot water during heating. The come-up time, or the time needed to reach the treatment temperatures, was approximately 6 s and was excluded from the heating time. Duplicate samples were removed from the water bath at different time intervals and immediately plunged into an iced-water bath. The sampling time was adjusted according to the heating temperature. Each temperature and salt combination was repeated at least twice on separate trials.

2.4. Enumeration of L. monocytogenes

Heat-treated and control samples were aseptically opened, added with 9 ml of PW, and homogenized for 2 min on each side at the maximum speed in a stomacher (Model BagMixer 100W, Interscience Co., France). After homogenization, a small volume (0.1 ml) of the liquid portion of the samples was withdrawn and plated, either directly or after serial dilutions, onto TSA/R plates in duplicate. When thermal treatment resulted in low bacterial counts, the surviving cells were recovered by plating 1 ml of undiluted samples onto TSA/R plates (3×). The plates were kept at room temperature for 2 h to allow the heat-injured cells to resuscitate, and then incubated at 37 °C for 48 h. The background microflora in the samples was suppressed by rifampicin in TSA/R plates and only rifampicin-resistant *L. monocytogenes* cells were recovered after incubation. The colonies were counted and recorded as log CFU/g.