Journal of Food Protection, Vol. 67, No. 2, 2004, Pages 383–386

Research Note

Thermal Inactivation of *Listeria innocua* in Salmon (*Oncorhynchus keta*) Caviar Using Conventional Glass and Novel Aluminum Thermal-Death-Time Tubes

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MS 03-21: Received 21 January 2003/Accepted 27 May 2003

ABSTRACT

Differences in the come-up times and thermal inactivation parameters of *Listeria innocua* in salmon (*Oncorhynchus keta*) caviar containing 2.5% salt using conventional thermal-death-time (TDT) glass tubes and a novel aluminum tube were tested and compared. Generally, the come-up times and decimal reduction times (*D*-values) were shorter and the change in temperature required to change the *D*-value (*z*-value) was longer in the aluminum than in the glass tubes. The *D*-values at 60, 63, and 65°C for the aluminum TDT tubes were 2.97, 0.77, and 0.40 min, respectively, and for the glass TDT tubes, these values were 3.55, 0.84, and 0.41 min. The *z*-values were 5.7°C in the aluminum and 5.3°C in the glass. Because of the shorter come-up time, the aluminum TDT tubes may provide a more precise measurement of microbial thermal inactivation than the glass TDT tubes, particularly for viscous materials, solid foods, and foods containing particulate matter.

The reported values for the thermal inactivation of pathogens in foods can vary widely. Some of this variance may be because of a failure to use proper kinetic models *(18, 19).* However, some differences may result from experimental methodology. Often, little attention is paid to the design of a thermal-death-time (TDT) tube type, which may affect the come-up time, heating uniformity, or heat distribution during thermal inactivation experiments. These factors could, in turn, affect the accuracy of the calculated decimal reduction times (*D*-values) and the changes in temperature required to change the *D*-values (*z*-values). Different types of TDT tubes are used for the determination of *D*- and *z*-values. The National Advisory Committee on Microbiological Criteria for Foods recommends sterile glass TDT tubes (9-mm inside diameter and 15-mm length) for determining the thermal inactivation kinetics of *Listeria monocytogenes* in foods *(2).* Yet, to our knowledge, no one has studied the impact of TDT tube type on the thermal inactivation kinetics of microorganisms in foods. Other devices are used, particularly for viscous or semisolid materials. For example, sterile glass TDT tubes (10 by 100 mm) have been used to predict *D*- and *z*-values in ham *(3).* Also, the heat resistance of *L. monocytogenes* in surimi-based imitation crabmeat and vegetables has been determined by the use of polyester pouches (10.16 by 10.16 cm) *(15, 16).*

Although widely differing values are reported in published studies for the heat resistance of *L. monocytogenes,* a *D*-value of 0.2 min at 70°C and *z*-values in the range of 5 to 8°C are typical *(6).* A $D_{60°C}$-value of 1.82 min and a *z*-value of about 5.1°C were obtained for *L. monocytogenes (3).* A $D_{66°C}$-value of 0.4 min and a *z*-value of 5.8°C were obtained for a stationary-phase *L. monocytogenes* in surimi-based imitation crabmeat *(16).* Ready-to-eat fish products can be contaminated with *L. monocytogenes (11).* This microorganism is difficult to control since it can be reintroduced into the processing environment, and it poses a risk for postprocessing contamination *(6, 22). L. monocytogenes* is a psychrotrophic pathogen, posing a food safety threat in refrigerated foods *(20).* Refrigerated storage alone is inadequate for controlling the growth of *L. monocytogenes (5, 7). L. monocytogenes* can flourish on precooked, packaged crawfish tailmeat stored under refrigerated temperature for several days *(4).* High-market-value aquatic food products have been the target of increased regulatory scrutiny *(1, 10).* Although the Food and Drug Administration has dropped the concept of exceptionally lethal cooking processes *(7, 22),* the emphasis has shifted toward improved process control and a precise determination of the destruction of the target microbe. This is generally a process that yields a 6-D reduction in *L. monocytogenes* for aquatic food products *(7).* This has renewed interest in developing pasteurization processes for heat-labile, high-market-value aquatic foods.

Caviars are ready-to-eat aquatic food products made by brining and curing fish roe *(1).* Caviar is heat labile and difficult to pasteurize successfully. It can only be heated to a temperature of 70°C without the eggs becoming dull and losing their color *(21).* Irreversible protein denaturation occurs between 70 and 80°C. Salmon caviar or ikura is most

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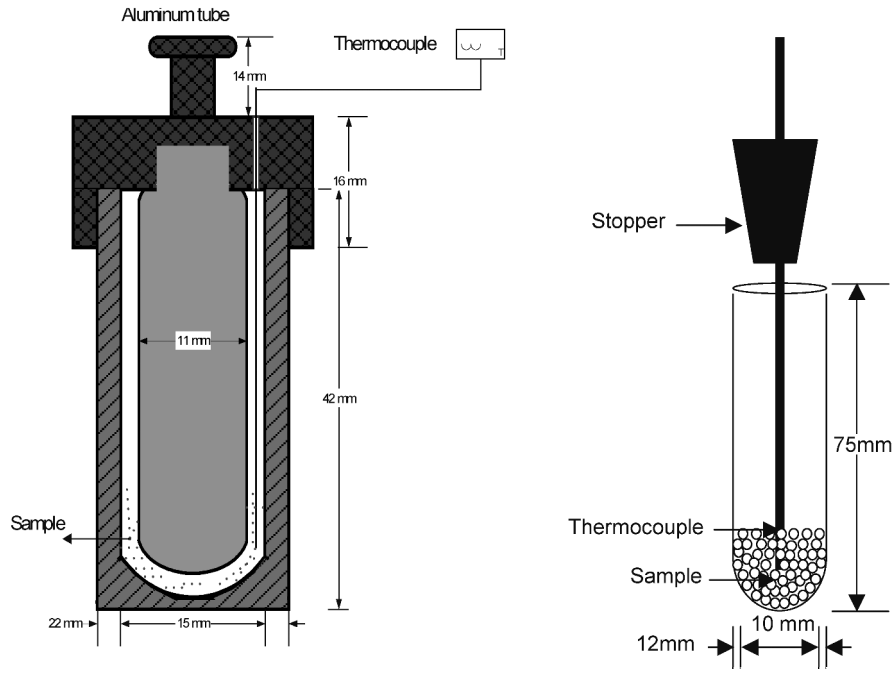

FIGURE 1. *Schematic representation of the glass (right) and aluminum (left) thermal-death-time tubes.*

commonly made from chum (*Oncorhynchus keta*) or pink (*Oncorhynchus gorbuscha*) salmon. Salmon caviars range from 20 to 38% protein and from 10 to 20% crude lipid *(1, 8, 9),* with a water activity value of 0.96 to 0.98, a pH value of 5.5 to 6.7, and a salt content from 2 to 5% *(1).* The main objective of this study was to determine the thermal inactivation properties of *Listeria innocua* in salmon caviar. A second objective was to compare the performance of a novel aluminum TDT device with conventional glass TDT tubes.

MATERIALS AND METHODS

L. innocua is a nonpathogenic organism that exhibits a response equivalent to *L. monocytogenes* for many physiochemical processing treatments, including its response to heat treatment *(12). L. innocua* ATCC 51742 was used in lieu of *L. monocytogenes* in this study. Presumptive *Listeria* sp. colonies were streaked for purity from brain heart infusion agar slants on *Listeria* PALCAM medium base (Difco Laboratories, Sparks, Md.) that was supplemented with a Bacto PALCAM antimicrobic-selective supplement. A sterile loop of the selective media was transferred to 50 ml of tryptose broth (Difco) in 250-ml screw-cap dilution bottles. Cells in the stationary phase were used for the thermal inactivation experiments, because this phase is the most thermally resistant *(14).* One milliliter of the 24-h-old culture was used to inoculate ca. 15.00 g of salmon caviar.

For thermal inactivation experiments, thawed salmon (*O. keta*) roe (Southern Southeast Regional Association, Ketchikan, Alaska) containing 2.5% salt was transferred aseptically to a 100-ml sterile beaker and inoculated with *L. innocua* (ca. 10^7 CFU/g); this was then mixed vigorously with a sterile spatula to obtain a uniform cell distribution. One gram of the inoculated caviar was placed inside sterile glass culture tubes (10-mm outside diameter and 75-mm length) or aluminum TDT tubes. Figure 1 shows the dimensions of the glass and novel aluminum TDT tubes. The filled tubes were kept in an ice bath at 0.0 ± 0.2°C for 30 min. Thermal inactivation was performed with a circulating water bath (Digi-bath Laboratory Devices Inc., Holliston, Mass.). The temperature of the water bath was controlled at 60, 63, and 65 ± 0.2°C. The TDT tubes were submerged completely in the water bath at the specified treatment temperature. The come-up time was defined as the time required to bring the material at the coldest point of the TDT tubes to the specified treatment temperature after the tubes had been submerged in the water bath.

A 1.00-g inoculated sample (±0.02 g) that was not exposed to heat served as a control. A sample was removed immediately after the come-up time was reached; this time was designated time zero. TDT tubes were removed from the water bath at intervals of 1.5, 0.5, and 0.33 min at 60, 63, and 65°C, respectively. After removal, the samples were promptly immersed in an ice bath at 0.0 ± 0.2°C.

The temperature of the inoculated TDT tubes was monitored with a subminiature type T thermocouple (Barnant 115 Model 600-1020, Barnant Co., Barrington, Ill.) placed in the center of the caviar in the TDT tubes. The temperature was recorded during the experiment by a data acquisition system (Analog Connection ACPC, Strawberry Tree Inc. Computer Instrumentation and Controls, Sunnyvale, Calif.) and process control software.

The *L. innocua* survivors were enumerated by diluting each heat-treated sample in 99 ml of sterile 0.1% peptone in a 250-ml screw-cap dilution bottle. The homogeneous dilution was serially diluted in 0.1% peptone and then plated by the overlay method *(13)* to determine the number of survivors. This overlay method was designed specifically to improve the recovery of heat-injured cells. Tryptic soy agar (TSA) pour plates were incubated at 37°C for 2 h to allow the injured *Listeria* cells to repair and resuscitate; then, about 7 ml of the *Listeria*-selective PALCAM agar containing the antimicrobic supplement was overlaid onto the TSA. The plates were incubated for an additional 22 h at 37°C.

The log numbers of the survivors at each temperature were plotted against the time. The best-fit line was extrapolated, and the *D*-values were determined. The *z*-values were determined by plotting the calculated log *D*-values against the corresponding temperatures. Each single number is an average of at least three replicate experiments. The standard deviation was determined. Data were analyzed with a computer software package (SAS Institute, Cary, N.C.). For the analysis of variance, Fisher's least significant difference test was used.

TABLE 1. *Come-up times and heat resistance of* Listeria innocua *in salmon caviar heated in glass and aluminum thermal-death-time tubes*

Temp (°C)	Come-up time (s)[a]		*D*-value (min)[a]	
	Glass	Aluminum	Glass	Aluminum
60	181.7 ± 10.4 A[b]	82.7 ± 2.5 B	3.55 ± 1.11	2.97 ± 1.12
63	183.3 ± 10.4 A	86.7 ± 2.9 B	0.84 ± 0.13	0.77 ± 0.13
65	188.3 ± 5.6 A	90.0 ± 0.5 B	0.41 ± 0.07	0.40 ± 0.01

[a] Average ± standard deviation of three measurements.
[b] Values with different letters in the same row are significantly different ($P < 0.05$).

RESULTS AND DISCUSSION

To our knowledge, no previous studies have evaluated the inactivation of pathogenic microorganisms in caviar. Because of the high heat resistance of *Listeria* sp., its ability to grow at refrigeration temperatures, the ubiquitous occurrence of the microbe, and the current zero-tolerance standard in Food and Drug Administration–regulated foods, control of this organism is vitally important for refrigerated foods, including salmon caviar. In this study, the thermal inactivation of *Listeria* was dependent on the type of TDT tube used. The come-up times were significantly shorter in the aluminum TDT tubes than in the glass tubes ($P < 0.05$) (Table 1). The design of the tube has a marked effect on the come-up time. This difference cannot be accounted for by differences in the thermal conductivity of the glass or aluminum from which the TDT devices were constructed. The more likely explanation is that the conductive heating of a thin sample layer (ca. 2 mm; aluminum TDT tube) is more rapid than the heating of a radial plug of the sample (ca. 10 mm; conventional glass TDT tube) (Fig. 1).

The sample thickness was greater in the glass tube than in the metal tube (Fig. 1). The cylindrical insert of the aluminum tube helped distribute the sample on the heating surface, thus reducing the sample thickness and increasing the surface area exposed to the heating medium compared to the glass TDT tubes. The aluminum insert was at the same temperature as the water bath at the end of the come-up time. Heat was diffused from the water bath through the tube wall and from the tube closure through the tube insert and then into the sample. In contrast, the heat transfer into the glass tube was through the wall only. This resulted in more rapid heat transfer and consequently shorter come-up times for the aluminum TDT tubes. As an example, Figure 2 shows the heat penetration in the glass and aluminum TDT tubes at 60°C.

The *D*-values of *L. innocua* in salmon caviar heated in the glass and aluminum TDT tubes are presented in Table 1. As expected, the rate of thermal inactivation increased as the treatment temperature increased. The *D*-values were generally smaller for the aluminum than for the glass TDT tubes. Although there were differences in the *D*-values at 60 and 63°C, they were not significant ($P > 0.05$). The *D*-values at 63°C were in the range for *L. monocytogenes* at 62.8°C (0.58 to 1.22 min *(2)*). Additionally, the *D*-values

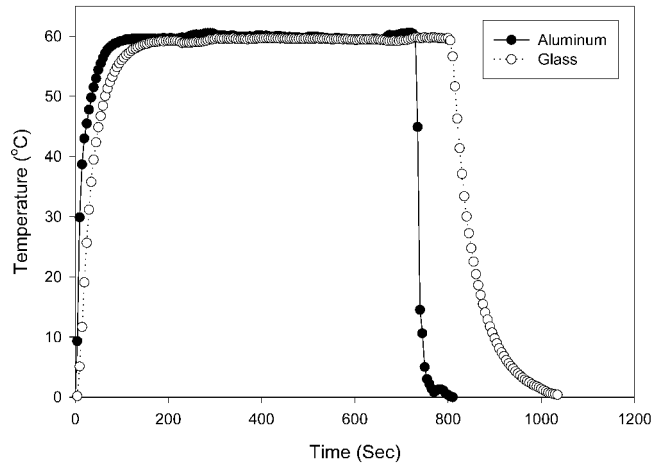

FIGURE 2. *Effect of the glass and aluminum thermal-death-time tubes on heat penetration in salmon caviar at 60°C.*

were comparable to those reported by Mazzotta *(16)* for *L. monocytogenes* in surimi-based imitation crabmeat at 62°C (2.1 min) and 66°C (0.4 min) that was heated in thin plastic pouches. However, the *D*-value at 64.4°C for *L. monocytogenes* in plain egg yolk heated by a submerged-vial technique was 0.44 min *(17)*. Although there are widely differing values reported for the heat resistance of *L. innocua*, *z*-values from 5 to 8°C are typical *(6)*. The *z*-values found for *L. innocua* in salmon caviar were 5.7 and 5.3°C in the aluminum and glass tubes, respectively (Fig. 3).

First-order thermal inactivation kinetics for *L. innocua* ATCC 51742 in salmon caviar (*O. keta,* 2.5% salt) from 60 to 65°C using conventional glass and newly designed aluminum TDT tubes were observed. The come-up times in the glass tubes were roughly two times longer than in the aluminum TDT tubes. These results were consistent with the reported values for *Listeria* sp. in other foods. The aluminum TDT tube provided a more rapid heat transfer and more even heat distribution than the glass tube. This was reflected in the shorter come-up times for the lower treatment temperatures. Even though there were no signif-

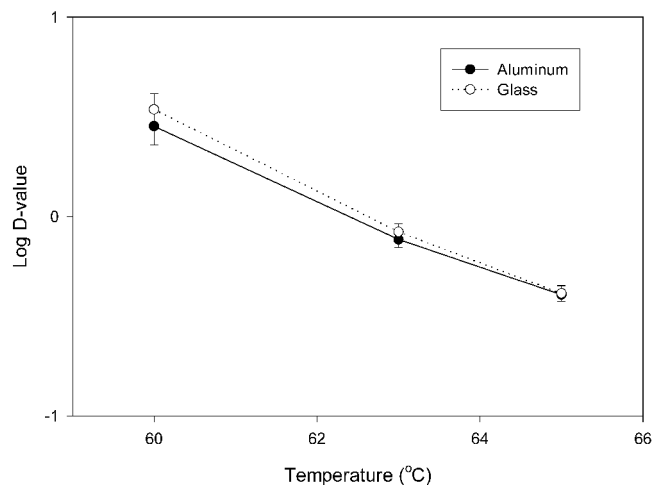

FIGURE 3. *Thermal-death-time curves for* L. innocua *in salmon caviar heated in the glass and aluminum thermal-death-time tubes.*

icant differences in the *D*-values between the glass and aluminum TDT tubes ($P > 0.05$), the calculated *D*-values were generally lower in the aluminum than in the glass tubes, indicating that some reported *D*-values for *Listeria* sp. may be too high. This novel aluminum TDT tube may provide more reliable thermal inactivation parameters than glass tubes and is particularly suitable for testing products that are delicate or heat sensitive. The findings of this study can be used to properly design a thermal pasteurization process for salmon caviar.

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